THESIS

ADDRESSING CONSTRAINTS TO RESTORATION OF HIGHLY DISTURBED ECOSYSTEMS AFFECTED BY CHEATGRASS INVASION AND SLASH PILE BURNING

Submitted by

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ABSTRACT

ADDRESSING CONSTRAINTS TO RESTORATION OF HIGHLY DISTURBED ECOSYSTEMS AFFECTED BY CHEATGRASS INVASION AND SLASH PILE BURNING

Chapter 1. In 2010, a study was established at an old field in northern Colorado to examine competition between the invasive annual grass cheatgrass (*Bromus tectorum* L.) and native ruderal species with similar traits. Cheatgrass and the sterile Triticale hybrid QuickGuard were broadcast seeded, and a seed mix of native ruderal species was rototilled into the top 10 cm of soil. The study design allowed for exploration of several hypotheses: (H1) that seeding native ruderal species would suppress cheatgrass establishment, (H2) that rototilling native ruderal species into soil would create a persistent soil seed bank, and (H3) that this soil seed bank would continue to reduce cheatgrass establishment in bare patches created by soil disturbances years after seeding. One year after initial seeding, a subset of plots was also drill-seeded with a native perennial mix. This was in order to examine a fourth hypothesis, (H4) that native ruderal species would facilitate the establishment of native perennial species, likely through the cultivation of beneficial mycorrhizal communities.

Results from the first two growing seasons showed that seeding native ruderal species suppressed biomass and density of cheatgrass better than Triticale. In 2018, I re-initiated the study in order to address the second, third, and fourth hypotheses. Initially, I collected soil samples from plots to examine the soil seed bank in a greenhouse study. I collected field biomass data in summer 2019. In fall 2019, I rototilled half of each plot to stimulate soil seed bank germination, and I sampled biomass again in summer 2020.

The greenhouse study showed viable soil seed banks of four species from the native ruderal seed mix, as well as continued presence of viable cheatgrass seeds in the soil. However, field biomass data in both 2019 and 2020 showed very low biomass of native ruderal species and no cheatgrass biomass. Neither year showed any significant difference between native ruderal biomass between plots where native ruderals

were and were not seeded. Furthermore, there was no significant difference in native ruderal species biomass between rototilled and undisturbed plots in 2020, suggesting that seedlings were failing to establish from the native ruderal soil seed bank after soil disturbance. Native perennial species produced no biomass in 2019 and an insignificant amount in 2020, so the study failed to support any facilitation effect of the co-occurrence of native ruderal species on native perennial establishment.

I concluded that the lack of plant establishment from the soil seed bank after disturbance was likely related to competition and soil legacy effects from *Agropyron cristatum* L. (Gaertn.) (crested wheatgrass), which dominated site biomass in 2019-2020. High grasshopper herbivory on native ruderal seedlings has been found by previous studies at the same site, and may also have impacted results in this study. Overall, this study found evidence that the native ruderal species studied can form persistent soil seed banks, and initial results suggested that they can also suppress cheatgrass growth. Future studies should avoid the confounding effects of highly competitive co-occurring species such as crested wheatgrass to determine whether this combination of competitiveness and persistence in native ruderal species can provide long-term suppression of cheatgrass.

Chapter 2. Slash pile burning is a common forest management practice throughout the western United States. It is used to reduce wildfire fuels after forest thinning projects. Slash pile burning heats soils to temperatures much hotter than wildfires, and may result in persistent patches of bare ground or invasive herbs. These patches are known as pile burn scars. In the montane *Pinus contorta* Douglas ex Loudon (lodgepole pine) forests of north-central Colorado, pile burn scars may persist for decades.

The process of ecological succession may be driven in part by plant-soil feedbacks whereby early seral species alter soil conditions in ways that benefit later seral species. If tree recruitment in pile burn scars is limited due to improper soil conditions, then perhaps seeding early seral understory plants into pile burn scars could ameliorate those conditions and improve lodgepole pine growth. To address this hypothesis, I conducted two greenhouse studies using soil samples taken from pile burn scars at two locations in Colorado. The first study involved pre-conditioning pile burn scar soil with one of three seed mix treatments for 14 weeks. The treatments were pre-conditioning with either a perennial grass mix, the

shrub *Arctostaphylos uva-ursi* (L.) Spreng., or nothing (unseeded). After pre-conditioning, I removed plants and transplanted a lodgepole pine seedling into each pot. I grew the lodgepole pine seedlings for 6 weeks, measuring diameter and height at the beginning and end of this period. From those measurements, I was able to calculate relative growth per tree in terms of stem volume for the purposes of comparing the effects of the treatments on lodgepole growth.

I chose the perennial grass mix to represent a typical forest revegetation seed mix. I was interested in testing the effects of bearberry pre-conditioning because prior studies have found that other *Arctostaphylos* species facilitate various pine species through shared mycorrhizal communities. However, such a relationship has not been examined for lodgepole pine and bearberry, which commonly grow together in Colorado. I hypothesized that bearberry would facilitate lodgepole pine growth specifically by promoting mycorrhizal communities that would benefit lodgepole pine. To examine this hypothesis, I also conducted a second greenhouse study using only bearberry or unseeded treatments for pre-conditioning. Within these two pre-conditioning treatments, I created an additional treatment level by promoting or discouraging mycorrhizal hyphal access to an extra reservoir of soil nutrients. I separated lodgepole roots from this extra reservoir with a fine mesh that would allow hyphae but not roots through, and periodically severed hyphae in half of the pots from both pre-conditioning treatments. I hypothesized that only bearberry treatments with intact hyphal networks would show benefits to lodgepole pine growth.

Results from the first greenhouse study showed that pre-conditioning soil did improve the relative growth rate of lodgepole pine stem volume relative to the unseeded treatment. However, which treatment showed significantly improved growth depended on the site from which the soil samples were collected. At the Crown Point site, only the bearberry treatment showed improved relative growth rate, and at the Fraser site, only the perennial grass treatment showed improved relative growth rate. In the second greenhouse study, only replicates from the Fraser site showed significant treatment differences in an ANOVA, and there were not significant differences between treatment pairs in the post-hoc pairwise comparisons.

Overall, the study did not find support for the hypothesis that soil conditioning with bearberry would improve lodgepole growth more than other treatments. Rather, pre-conditioning may improve lodgepole growth but which species provide the best results may depend on soil conditions. The hypothesis that bearberry facilitates lodgepole growth through a mycorrhizal plant-soil feedback was not directly supported either. A nonsignificant trend indicated that lodgepole pine grown in soil conditioned with bearberry may be more dependent on mycorrhiza for growth, but it did not indicate that this dependence actually resulted in improved growth when compared to the unseeded treatment.

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1. Introduction

1.1 Ecosystem Effects of Cheatgrass Invasion

Cheatgrass (*Bromus tectorum* L.) is a Eurasian annual grass that is highly invasive in western North America (Hulbert 1955). It originally entered the U.S. from the Pacific Northwest as a contaminant in wheat coming from Asia, and was sometimes planted intentionally as cattle forage (Mack 1981). Cheatgrass is now common across the arid and semiarid West, especially in sagebrush and bunchgrass steppe. By the late 20th century, up to 20% of these ecosystems were dominated by monotypic stands of cheatgrass (Knapp, 1996). Cheatgrass is likely the most prevalent invasive species in the Great Basin (Pellant 1990), and some authors call it the most widespread invasive plant in the entire western U.S. (e.g. Jones et al. 2015). Although it alters ecosystems in several significant ways, cheatgrass is particularly notable for increasing wildfire frequency and intensity in invaded areas (Stewart and Hull 1949, D'Antonio and Vitousek 1992, Knapp 1996, Knick and Rotenberry 1997) to levels beyond the tolerance of native species, particularly sagebrush (*Artemisia* spp.) (Pellant 1990). Balch et al. (2013) suggest that cheatgrass-invaded rangelands in the northern Great Basin burn up to four times more frequently than their uninvaded counterparts, while Whisenant (1990) and Brooks and Pyke (2001) concluded that fire return intervals in the Great Basin have shortened from 60-110 years to 3-5 years following cheatgrass invasion.

Cheatgrass invasion reduces biodiversity in ecological communities across multiple trophic levels. The Center for Science, Economics and Environment (2002) found that 20% of sagebrush steppe flora and fauna in the Great Basin are imperiled. This is related in part to the native flora's lack of tolerance to increased fire frequency after cheatgrass invasion, which has cascading effects on fauna (Chambers and Wisdom 2009). Sagebrush-dependent species such as the greater sage-grouse (*Centrocercus urophasianus*)

and Columbia Basin pygmy rabbit (*Brachylagus idahoensis*) have become endangered due to cheatgrass invasion and human activities (Davies et al. 2012). There are effects on soil microbial communities as well. Busby et al. (2013) found cheatgrass to be a poorer host for arbuscular mycorrhizal fungi (AMF) than big sagebrush (*Artemisia tridentata*). This helps explain why a cheatgrass-invaded grassland in Utah showed lower AMF diversity compared to uninvaded references (Hawkes et al. 2006). Other studies have suggested broader repercussions of cheatgrass invasion on microbial communities, including reduced soil fauna abundance (Belnap et al. 2005) and altered soil microbial community composition across trophic levels (Belnap and Phillips 2001). These alterations to soil microbial communities may reduce carbon sequestration in rangelands (Jones et al. 2015)

Cheatgrass invasion also has negative economic impacts. Cheatgrass provides much poorer cattle forage than most native bunchgrasses (Knapp, 1996). This can reduce profits for ranchers to the point that ranches are no longer economically viable (Maher et al. 2013). Because they share similar phenologies, cheatgrass is also a troublesome weed in winter wheat (*Triticum aestivum)* fields in the Intermountain West (Massee and Higgins 1977, Peeper 1984, Keren et al. 2015). Land managers spend large amounts of time and money on suppressing invasive annual grass invasion after wildland fires in the Great Basin (Davies et al. 2011). This is because fires promoted by cheatgrass invasion pose risk to human life, health, and property both directly and through increased postfire water runoff and erosion (Wilcox et al. 2012, Weltz et al. 2014).

One land management organization that spends massive resources to combat cheatgrass invasion is the Bureau of Land Management (BLM), which oversees the management of more acreage than any other federal land management agency (Vincent et al. 2017). The majority of the acreage under BLM management is western rangeland (Vincent et al. 2017). Between 1998 and 2002, the BLM purchased 1.3 million kg of seed annually (Vincent et al. 2017). Most of these seeds are used in postfire reseedings meant to suppress invasive annual grasses. Seeded species are mainly native perennial grasses and some native shrubs (Leger and Baughman 2015). In fact, between 1998-2002 only 1% of seeds purchased annually were native forbs (Shaw et al. 2005). However, large-scale reseedings of perennial grasses often result in low native perennial grass cover and high exotic annual grass cover (Pyke et al. 2013, Knutson et al. 2014). A

prime example is the BLM revegetation project after the 2007 Milford Flat Fire, the largest fire recorded in Utah since Anglo-American colonization. The fire ignited when lightning struck a cheatgrass-invaded shrubland within Milford Flat. The BLM spent $17 million on postfire seeding with the aim of stabilizing soil and minimizing exotic annual grass invasion. Species seeded included native and exotic perennial grasses but only one native forb species (*Kochia americana*). Five years later, rehabilitated and non-rehabilitated areas had similar levels of average perennial grass cover, at around 3%. Both were widely invaded by exotic species (Duniway et al. 2015).

So far, postfire rehabilitation of the Milford Flat Fire could be considered a massive expenditure of time and money with minimal return on investment. Results like these raise the question of whether changing the composition of restoration seed mixes in the Great Basin or elsewhere would improve outcomes. The Milford Flat Fire is by no means the only case of postfire rangeland seeding that failed to significantly reduce subsequent exotic annual invasion. Pyke et al. (2013) performed a meta-analysis of 19 postfire seeding efforts in rangelands worldwide and found that only 28% of these resulted in a reduction of invasive species. They suggested that low rates of seeded species establishment left communities vulnerable to invasion. Similarly, Knutson et al. (2014) analyzed 88 BLM postfire re-seeding efforts in the Great Basin between 1990 and 2003, and found that seeding native perennial grasses and native shrubs generally did not significantly increase native plant cover relative to untreated areas.

In part, previous restoration efforts in the Great Basin have focused on seeding perennial grasses because studies have found communities with high perennial cover to be more resistant to invasion (e.g. Chambers et al. 2007, Jessop and Anderson 2007, Whittaker et al. 2008, Chambers et al. 2009). While this may be true in relatively undisturbed settings, perennial bunchgrasses and shrubs are not well adapted to the highly disturbed sites the BLM targets for revegetation. Cheatgrass, on the other hand, has high plasticity of several important growth and reproduction traits that allow it to outcompete native perennial species on degraded sites. Firstly, when conditions are favorable it benefits from extremely high seed production and germination rates. Cheatgrass produces 5,000-17,000 seeds/m^2 annually (Humphrey and Schupp 2001, Griffith 2010, Stewart and Hull 1949), and more than 98% of viable cheatgrass seeds

typically germinate in field settings (Stewart and Hull 1949, Hulbert 1955, Steinbauer and Grigsby 1957, Humphrey and Schupp 2001). Cheatgrass typically produces seed even in unfavorable years, albeit at a reduced level (Mack and Pyke 1983, Rice and Mack 1991, Rice et al. 1992). The high percentage of germinated seeds may be due in part to its early germination relative to other species. Cheatgrass is a winter annual, which means that when water is available it can germinate in the fall or winter and develop an extensive root system when many native seeds are still dormant (Harris 1967, Dobrowlski et al. 1990). By doing so, it coopts shallow groundwater that might otherwise later be available for native species (Kulmatiski et al. 2006). This winter annual life cycle also means that cheatgrass usually senesces early relative to native species. After senescence, cheatgrass leaves a large amount of flammable dry standing litter (Klemmedson and Smith 1964, Young and Evans 1973, Knapp 1996, Brown et al. 2008), which provide interstitial fuel that allows wildfire to jump between native bunchgrasses, promoting the aforementioned increase in wildfire frequency and intensity (Pilliod et al. 2017). The abundance of litter may also explain the shift from mycorrhiza- to saprophyte-dominated microbial communities following cheatgrass invasion (Hawkes et al. 2006). Many native perennials have not set seed by the time cheatgrass litter sparks fires in mid-summer, leading to rapid replacement of these native species by cheatgrass in the soil seed bank (Taylor et al. 2014).

1.2 Disturbance, Cheatgrass Invasion, and Native Ruderal Species

If cheatgrass benefits from disturbance, then perhaps native species that are also well-adapted to disturbed areas could compete with it more successfully than native perennial grasses. In fact, several authors have suggested that the relatively low abundance and richness of native annual grasses in the Great Basin left a phenological niche open for cheatgrass to exploit (Young and Evans 1973, Knapp 1996, Hawkes et al. 2006). Still, there are a variety of ruderal or early seral forbs and grasses that are native to the western U.S. and might compete effectively with cheatgrass. The pool is larger if the definition of native is expanded to a regional scale, and if species with debated origins are included. This approach might be controversial,

but it would be worth seeding some of these species in restoration projects if they could competitively suppress cheatgrass without excluding other native species.

Prior work has suggested that native ruderal species may compete more effectively with cheatgrass than native late seral species (Daehler 2003, Leger et al. 2014, Barak et al. 2015). The species seeded in these studies shared key traits with cheatgrass, such as high germination rates, fall germination, and rapid growth. Unlike cheatgrass, they have coevolved with late-seral native species as part of a successional process, and may play an important role in early community assembly even if they are absent later (Leger et al. 2014). In other words, they may suppress cheatgrass and thereby allow ecosystems to shift to states where succession proceeds towards the establishment of the perennial bunchgrasses and shrubs that were common in the historical reference conditions. The ability of native ruderals to facilitate native perennial grass establishment in prior studies may relate not only to direct competitive suppression of cheatgrass, but also to facilitation of arbuscular mycorrhizal fungi (AMF) communities.

As an invasive, cheatgrass reduces the diversity of native AMF communities (Evans et al. 2001, Hawkes et al. 2006). There are two probable reasons for this: one, cheatgrass reduces the diversity of native mycorrhizal host plants in invaded areas, and two, cheatgrass itself is not an obligate AMF host. Cheatgrass may also promote a shift from more AMF-dominated towards saprophyte-dominated soil communities (Weber et al. 2015). Belnap and Phillips (2001) theorized that this might be both because cheatgrass makes few mycorrhizal associations and because it produces large amounts of litter annually. Seeding native arbuscular mycorrhizal ruderal plant species such as *Aristida purpurea* Nutt. (purple threeawn)*, Helianthus annuus* L. (common sunflower)*,* and *Sphaeralcea coccinea* Nutt. (Rydb) (scarlet globemallow) may help reestablish native AMF communities (Cameron 2010, Busby et al. 2013). Restoring AMF community functions in soil benefits later successional native perennial species (Busby et al. 2013), the establishment of which is often considered a benchmark of restoration success.

1.3 The Ecological Role of Soil Seed Banks

Native ruderal plant species are likely to be more advantageous in restoration projects if they have persistent soil seed banks. The soil seed bank is the collection of living plant seeds present in the soil of a given site. Mature grassland communities often feature soil seed banks with significantly different species composition from the aboveground vegetation (Chippindale and Milton 1934, Champness and Morris 1948, Douglas 1965, Major and Pyott 1966, Johnston et al. 1969, Roberts 1972, Hayashi and Numata 1975, Saatkamp et al. 2014). In undisturbed sections of native grasslands and shrublands, standing vegetation is typically dominated by slow-growing late seral species that rely on succulence, woodiness, clonality, underground storage organs and associations with soil microbes to withstand temporal changes in their environments (Rees 1996, Tuljapurkar and Wiener 2000). On the other hand, soil seed banks in these same ecosystems are usually composed predominantly of ruderal or annual species that can effectively exploit the nutrient flushes associated with soil disturbances by germinating, growing and reproducing quickly (Chesson and Warner 1981, Thompson and Hodkinson 1998, Angert et al. 2009).

Because cheatgrass is such a prolific seed producer, suppressing it via restoration seeding can require many consecutive years of seeding. Taking advantage of native ruderal species that both compete effectively with cheatgrass and have persistent soil seed banks could make restoration projects more efficient. Years or decades after initial seeding, these species could germinate when disturbance creates gaps in vegetation that would otherwise be quickly occupied by cheatgrass. While not all ruderal species have persistent soil seed banks, this trait is thought to be common among ruderal species since it can allow them to persist in ecosystems even when the conditions that favor their aboveground growth may occur unpredictably in time and space. Ruderal species, including cheatgrass, are adapted to recurring soil disturbance cycles generated by any process that produces bare soil (Bossuyt and Hermy 2001). This could include grazing, small mammal burrowing, drought, fire, and many other processes. It could also include anthropogenic disturbance.

Because soil seed bank viability declines over time, and because cheatgrass produces so many seeds, using native ruderal seeds is likely most effective with high seeding rates. Even though cheatgrass

produces 5000-17000 seeds m^2 with up to 98% germination (Stewart and Hull 1949, Steinbaur and Grigsby 1957, Humphrey and Schupp 2001, Griffith 2010), many rangeland revegetation projects in the western U.S. drill-seed restoration seeds at rates of 200-800 seeds m^2.

1.4 Previous study: seeding native ruderal species vs. cheatgrass (2010-2012)

Kieffer-Stube (2012) investigated whether native ruderal species could effectively compete against cheatgrass in an old field in north central Colorado. I continued this work using the same plots, which were established on Colorado State University's Gabbard-Routledge Property. This property is a former ranch near Waverly, CO, approximately 16 km north of the Colorado State University campus in Fort Collins. The ranch was heavily seeded with exotic pasture grasses in the 1950s and 1960s, including *Agropyron cristatum* L. (Gaertn.) (crested wheatgrass). Crested wheatgrass still dominates the study area, along with the native shrub *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom and Baird (rubber rabbitbrush).

Treatments were established in 2010. Vegetation was removed from the study area in two steps. First, shrubs were removed by chaining, and then remaining herbaceous vegetation was cleared by mowing, ripping, and rototilling. The field site was divided into fifty 3- x 3-m plots, with each plot receiving one of six seed mix treatments. These were: a native ruderal mix (NR) (Table 1.1), cheatgrass (BT), a sterile wheat hybrid called Quickguard (SW), native ruderal mix plus cheatgrass (NR+BT), sterile wheat plus cheatgrass (SW+BT) and a control (US). There were 10 replicate plots each of BT, NR+BT, SW+BT, and US, and 5 replicates each of NR and SW. Both the NR and NR+BT plots received 20,000 Pure Live Seed (PLS)/m^2 of native ruderal seed mix, while the BT, NR+BT, and SW+BT plots each received 7,650 PLS/m^2 of cheatgrass seed. The native ruderal seed mix was broadcast and then rototilled into the top 10cm of the soil, and cheatgrass and sterile wheat were subsequently broadcast seeded. It was hypothesized that this would create a persistent soil seed bank of native ruderal species. Additionally, a native perennial mix was drill-seeded into some of the plots in 2011, 1 year after the native ruderal seeding. A set of 5 plots was randomly chosen from each of the NR+BT, BT, SW+BT, and US treatments for drill-seeding. NR and SW did not

receive the native perennial mix. This drill-seeding treatment allowed for future study of potential native ruderal facilitation of native perennial establishment.

Table 1.1 Composition of the NR (native ruderal) seed mix. This mix was broadcast seeded at the study site in Larimer County, CO in 2010, and then rototilled to a depth of 10 cm into the soil. These species are native to the High Plains, although there is some debate over the native range of *Chenopodium album*. The species also share competitive traits with the invasive annual grass cheatgrass (*Bromus tectorum*). The relevant shared traits are listed under 'Ruderal Traits'.

Genus and species	Common Name	Life History	Functional Group	Ruderal Traits	**Seed Rate (PLS/m^2)**
Amaranthus retroflexus L.	Redroot amaranth	Annual	Forb	Fast-growing, fall germination	2800
Aristida purpurea Nutt.	Purple three-awn	Annual	Grass	Fast-growing, mycorrhizal	1400
Chenopodium album L.	Lambsquarters	Annual	Forb	Fast-growing, high seed production	2500
Cleome serrulata Pursh	Rocky Mountain beeplant	Annual	Forb	Fast-growing, high seed production	2400
Festuca octoflora Walter	Sixweeks fescue	Annual	Grass	Fall germination, mycorrhizal	2400
Gaillardia aristata Pursh	Blanketflower	Annual	Forb	Fast-growing, mycorrhizal	1700
Helianthus annuus L.	Common sunflower	Annual	Forb	High seed production, mycorrhizal	2000
Erysimum capitatum (Douglas ex. Hook) Greene	Sanddune wallflower	Biennial/Perennial	Forb	Fast-growing	2400
Sphaeralcea coccinea Nutt. (Rydb.)	Scarlet globemallow	Biennial/Perennial	Forb	Mycorrhizal	2400

Kieffer-Stube (2012) found that NR+BT plots showed reduced cheatgrass biomass compared to BT plots in the first growing season. Additionally, NR suppressed cheatgrass biomass more than sterile wheat during that growing season. The biomass effect was not present in the second growing season, likely due to drought. However, in the second year NR+BT showed reduced cheatgrass density compared to BT and BT+SW plots, which were not significantly different from each other.

In my study, which took place over the 8th, 9th, and 10th seasons after seeding, I returned to these plots to see if the early results continued to apply, to examine some additional questions. My study addressed five overarching questions: (1) *is there still a viable soil seed bank of the native ruderal species from the NR mix*? (2) *are native ruderal species still present in the aboveground vegetation? (3) have native ruderal species suppressed cheatgrass?* (4) *will the presence of a native ruderal soil seed bank limit the re-establishment of cheatgrass after a soil disturbance*? And (5) *have native ruderal species facilitated the establishment of native perennial species?*

2. Greenhouse Study

2.1 Greenhouse Study Questions and Hypotheses

To address the Question 1 (whether there is still a viable soil seed bank of native ruderal species), I formed the following hypotheses:

*Hypothesis 1A***:** Soil seed banks from the plots seeded with the native ruderal seed mix (NR+BT and NR) will contain a significantly greater abundance of NR species than soil seed banks from the plots not seeded with the native ruderal seed mix (BT, SW, SW+BT and US).

*Hypothesis 1B***:** Soil seed banks from the plots seeded with cheatgrass (BT, NR+BT and SW+BT) will contain a significantly greater abundance of viable cheatgrass seeds than soil seed banks from plots not seeded with cheatgrass (NR, SW and US).

While this study is not an investigation of cheatgrass soil seed banks, hypothesis 1B was a prerequisite to answer Questions 3 and 4. If cheatgrass was not present in the soil seed bank, I would have had to reseed it to answer those questions. The first step I took to evaluate Hypotheses 1A and 1B was to undertake a greenhouse study, using soil samples collected from the field site in the fall of 2018. I potted the soil samples in a greenhouse setting and observed which plant species germinated from the soil seed bank. This pilot study was an efficient way to determine if it was worth investigating the other questions. If the native ruderal were not present in the soil seed bank, then there would be no need for a field study.

2.2 Greenhouse Study Methods

In November 2018, I collected soil samples from the field plots. My samples were 10.5 cm deep soil cores, taken using a soil corer. To reduce the effects of in-plot variability on my results, I sampled systematically along transects (Figure 1.1). To increase statistical power for later analyses I randomly selected the distance from the plot boundary to the 1st transect. I laid out the remaining transects and sample points systematically at 0.4 m intervals. For each plot, I collected 5 sub-samples along 5 transects, for a total of 25 sub-samples per plot. Because I was interested between-plot rather than within-plot variation (to compare treatments), I pooled all the sub-samples for each plot into single plot samples, giving me 50 samples total.

To examine the seed present in the soil samples, I potted each composite plot sample into its own 0.3 m x 0.3 m tray, on top of a generic potting soil. I placed all 50 trays onto heating mats on a 12h day/night heating cycle and set the mats to heat the soil to 23°C during the day and to turn off at night. This simulated outdoor day/night temperature cycling, which stimulates seed germination in the fall or spring (Baskin & Baskin 2014). The day/night lengths and temperatures I chose were based on a literature review of the germination requirements for the species from the NR seed mix. I also misted trays daily and included 10 control trays that contained only potting soil and no field soil to account for contamination from seed rain in the greenhouse. To minimize the effects of this contamination, I randomly rearranged the trays every two

weeks. Control trays were not moved during the study. As species germinated, I identified, recorded, and removed them. After 4 months, I stopped the experiment.

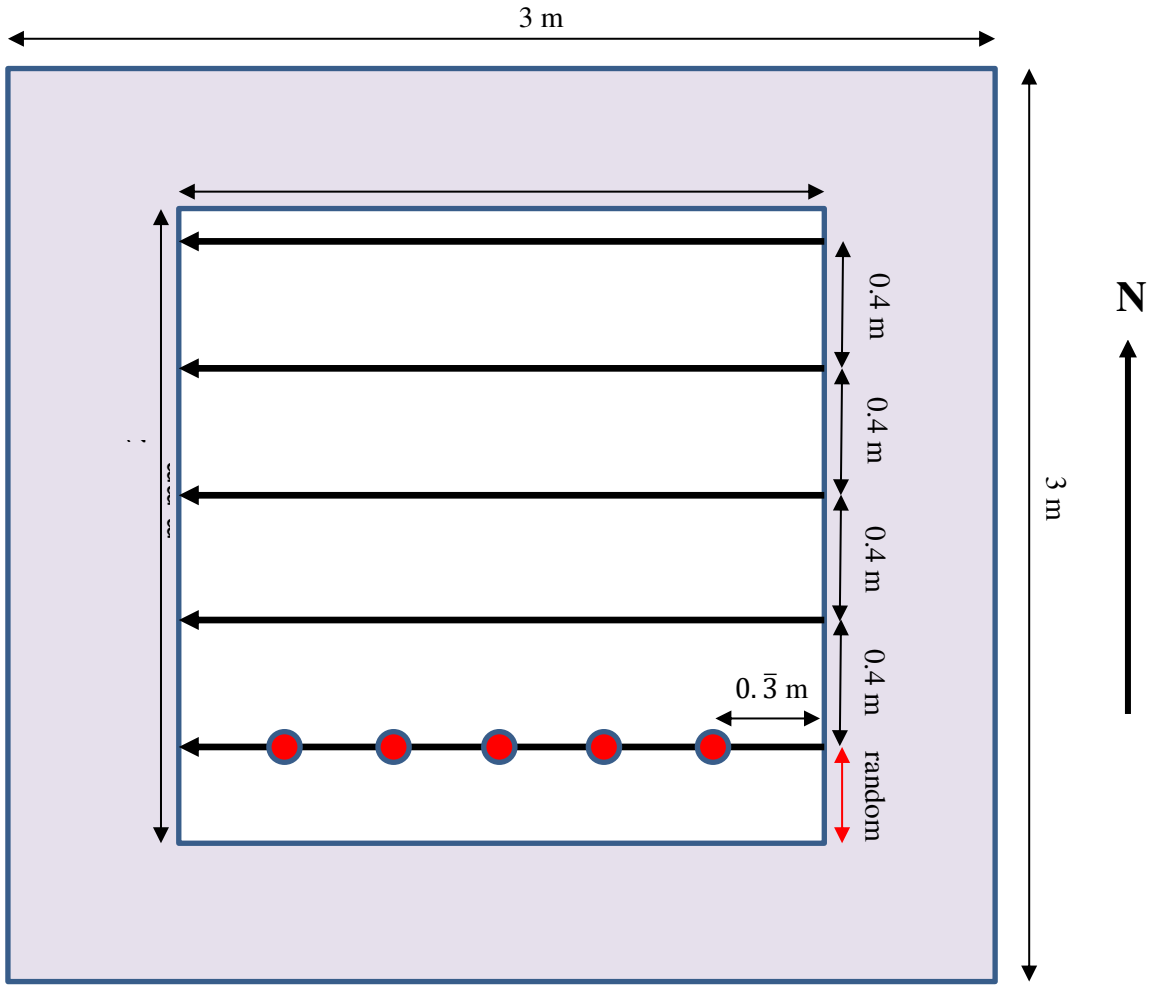

Figure 1.1 Soil sampling methods for the greenhouse study. The figure shows one of the 3x3 m plots initially established in 2010 (shown in purple). Within each plot, I sampled from a 2x2 m sub-plot (shown in white), leaving a 0.5 m buffer from the plot margin. Unidirectional arrows represent soil sampling transects. The sample points along one example transect are shown as red dots. They were spaced at 0.33 m from each other or the sub-plot boundary. The same pattern was repeated for the other transects. The initial transect is a random distance between 0.00-0.40 m from the plot margin, as shown by the "random" arrow. This was done for statistical randomization. I established each remaining transect 0.4 m from the last sampled transect.

2.3 Greenhouse Study Results and Discussion

The greenhouse study showed persistent soil seed banks for the NR mix species *Amaranthus retroflexus, Chenopodium album, Erysimum capitatum,* and *Helianthus annuus*. A two-sample t-test assuming unequal variance showed that NR species density was significantly higher in plots where NR species were seeded (treatments NR, NR+BT) (Figure 1.2). This suggests that the NR species present do not represent volunteers, but rather seeds from the initial study or descendants of the plants those seeds produced.

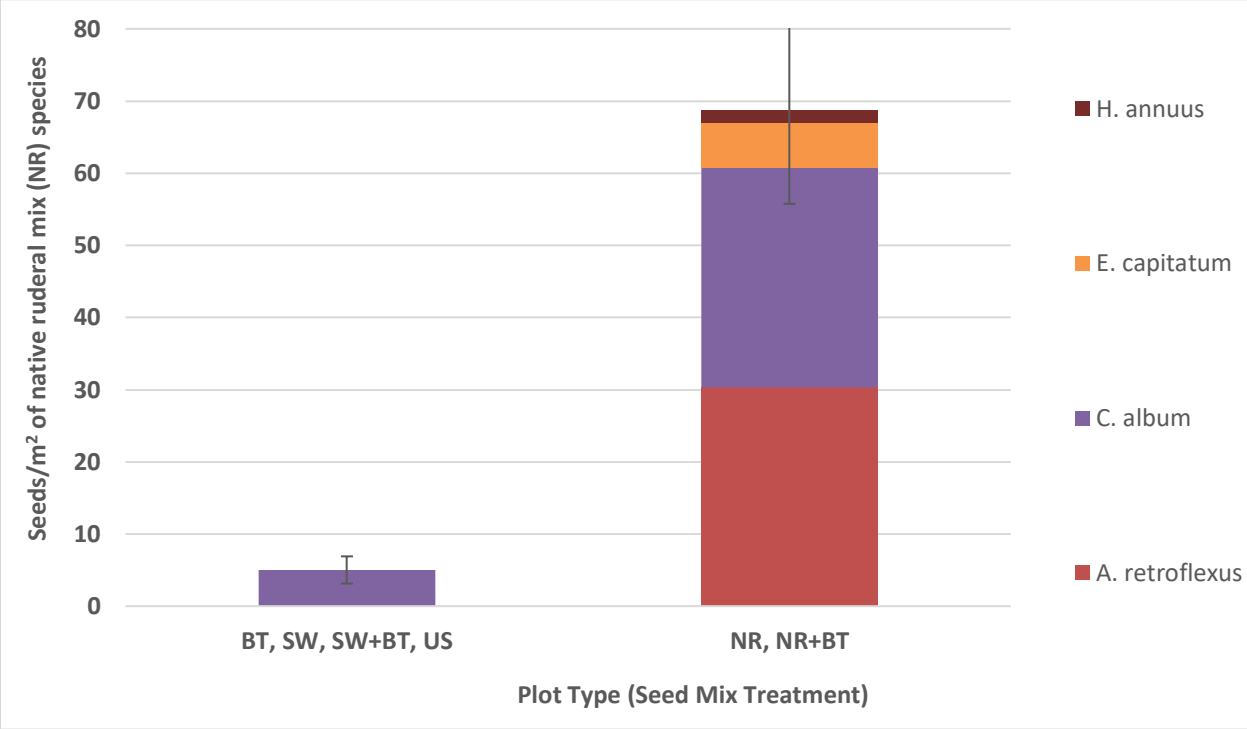

Figure 1.2 Eight years after seeding, native ruderal (NR)-seeded plots (treatments NR, NR+BT) have significantly higher estimated soil seed bank densities of NR species compared to plots where NR was not seeded (BT, SW, SW+BT, US). Soil seed bank densities were based on counting seedlings germinating from soil samples potted in a greenhouse study. NR species that were present in the soil seed bank included *Helianthus annuus, Erysimum capitatum, Chenopodium album,* and *Amaranthus retroflexus.* Of the NR species in the soil seed bank, only *Chenopodium album* germinated in plots where it was not seeded. Error bars show ± standard error for seeds/m^2 of all NR species. A two-sample t-test for a sample with unequal variance showed that plots seeded or not seeded with NR species had significantly different densities of NR species (p-value = 0.0002). This suggests that the NR seeds present in the soil samples I collected were either seeded for this study or represent descendants of seeded individuals.

The results further suggest that even the NR seeds present in plots where they were not seeded may be descendants of seeded individuals. *Chenopodium album* was the only seeded native ruderal that occurred outside of NR-seeded plots. Figure 1.3 shows that whenever *C. album* seeds did germinate outside of seeded plots, it was adjacent to NR-seeded plots that also contained *C. album* seeds. This suggests that *C. album* occurring in plots where they were not seeded were likely the progeny of seeded *C. album*, rather than passive recruits from outside of the study area. *C. album* produces large numbers of small, light seeds (Grundy et al. 2004, Ackerfield 2015), so aerial dispersal between adjacent plots is probable.

3. Field Study

3.1 Field Study Questions

Based on the results of the greenhouse study, I concluded that there was a viable native ruderal soil seed bank at the site. To address my remaining questions, I decided to conduct a field study. These questions were:

Question 2: Will native ruderal species establish from the soil seed bank after a disturbance?

Hypothesis 2: If half of each plot is subjected to a soil disturbance, the disturbed halves of NR-seeded plots will show greater increase in NR biomass than the undisturbed halves of NR-seeded plots.

Hypothesis 2 is based on the ecological theory that ruderal species are adapted to disturbance. Confirming this hypothesis would also support the idea that native ruderal soil seed banks could compete with cheatgrass in disturbance gaps even 8-9 years after original seeding.

Question 3: Is there still an aboveground community of the native ruderal species that were seeded?

Hypothesis 3: There will be more biomass of NR species in plots where the NR mix was seeded (NR and NR+BT) relative to plots where the NR mix was not seeded (BT, SW, SW+BT, US).

Question 4: Have native ruderal species suppressed cheatgrass in field plots?

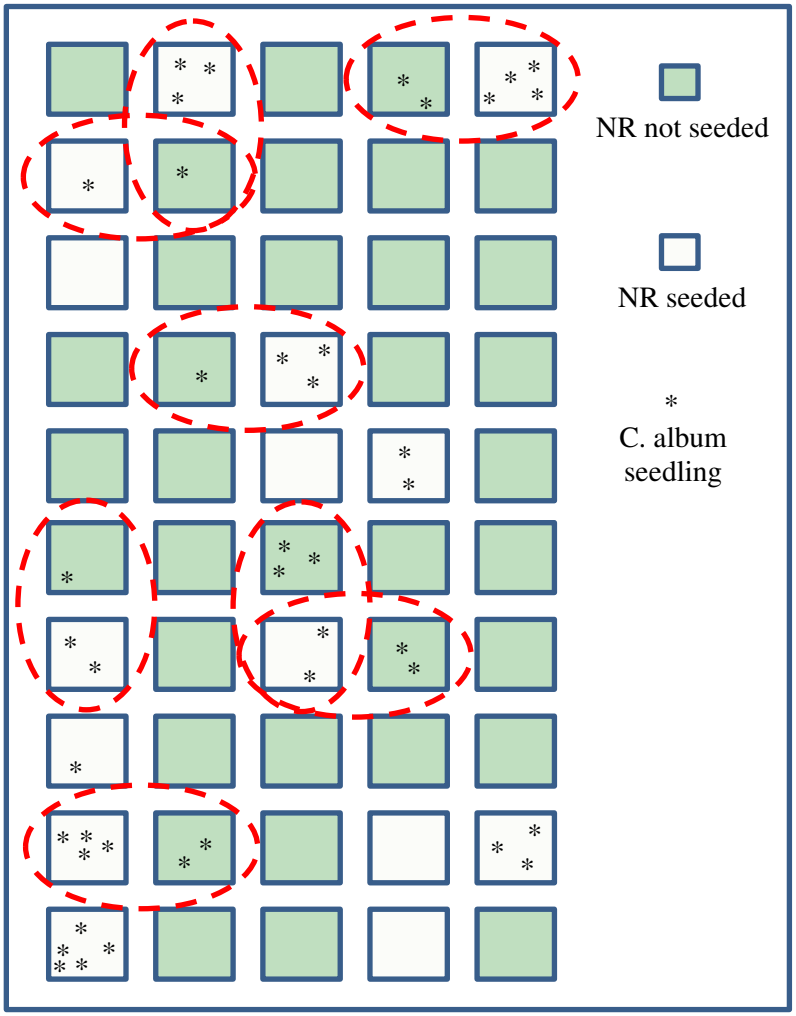

Figure 1.3 Map of plots where *Chenopodium album* seeds were found in 2018, 8 years after being sown. *Chenopodium album* seeds detected in plots where they were not seeded were always adjacent to plots where *C. album* seeds were sown in 2010 (NR-seeded plots). This suggests that the presence of *C. album* in plots where it was not seeded likely resulted from seed dispersal by individuals that were seeded in the initial study. *C. album* was the only seeded native ruderal that occurred outside of NR plots.

*Hypothesis 4A***:** Nine and ten years after seeding, plots seeded with both the NR mix and cheatgrass (NR+BT) will have significantly lower cheatgrass biomass than plots seeded with only cheatgrass (BT) or sterile wheat plus cheatgrass (SW+BT).

*Hypothesis 4B***:** Plots seeded with the NR mix will have lower cheatgrass biomass relative to unseeded control plots (US) or plots seeded with sterile wheat (SW).

I separated my hypotheses for question 4 into 4A and 4B because some treatments received cheatgrass seed and some did not. Since the majority of cheatgrass seeds typically disperse within 1 m of the parent plant (Pyke and Novak 1994), the propagule pressure is likely to be much higher in the seeded 3- x 3-m plots than in unseeded plots. Thus, hypothesis 4B is more likely to be true than hypothesis 4A.

*Question 5***:** Will the native ruderal soil seed bank suppress cheatgrass establishment after a soil disturbance?

*Hypothesis 5A***:** Localized soil disturbance in half of each plot will result in lower cheatgrass biomass in disturbed portions of NR+BT plots than disturbed portions of BT or SW+BT plots.

Hypothesis 5B: Localized soil disturbance in half of each plot will result in lower cheatgrass biomass in disturbed portions of NR plots than disturbed portions of SW or US plots.

*Question 6***:** Have native ruderal species facilitated the establishment of native perennial species?

*Hypothesis 6***:** Plots seeded with NR mix will have higher biomass and diversity of seeded native perennial species than plots seeded with cheatgrass. NR + BT plots will be intermediate.

3.2 Field Study Methods

To evaluate these hypotheses, I collected biomass samples from plots in July 2019. I sampled biomass of all species present in the plots using four 0.25- x 0.75- m frames in the 2- x 2-m sub-plots described in Figure 1.1. Each plot was split into a north and south half. I sampled two frames in the north half of each sub-plot, and two in the south half of each. This meant that I could return the following summer

after the rototilling to get a before/after comparison of both the disturbed and undisturbed halves of each plot. I sampled only current year's growth for perennial species.

For the rototilling, I used a randomized split-plot design: the rototilling treatment was randomly assigned to either the north or south half of each plot regardless of the initial seed mix treatment. I rototilled the plot halves in October 2019.

In July 2020, I returned to the plots to sample biomass a second time. The procedure was the same as in 2019, except that I changed the placement of the sampling frames (while maintaining two frames for each plot half) to avoid re-sampling areas sampled in the previous year.

For statistical analyses in the R language, I used RStudio software. I used non-parametric tests to evaluate hypotheses 2 and 3 due to large amounts of plots with zero values for NR biomass in both 2019 and 2020. To compare biomass in NR vs. non-NR plots for individual years, I used a one-sided Mann-Whitney U Test, with the alternative hypothesis that NR biomass would be greater in NR plots. When comparing change in NR biomass in rototilled versus undisturbed sub-plots from 2019 to 2020, I only examined NR-seeded sub-plots. I divided these into tilled and controlled treatments and analyzed each separately. To compare the paired before/after treatment data for each disturbance treatment, I used Wilcoxon signed-rank tests.

3.3 Field Study Results

In both 2019 and 2020, there was very low biomass of species from the native ruderal seed mix (NR) (Table 1.2). Of 30 NR-seeded subplots, only 3 showed nonzero changes in biomass between 2019 and 2020. Wilcoxon signed-rank tests also failed to support the hypothesis that soil disturbance (rototilling) would lead to higher native ruderal establishment. At a significance level of 95%, they showed no significant difference in median NR biomass from 2019 to 2020 in either tilled ($p = 0.19$) or control ($p = 0.98$) plots.

Table 1.2 Mean percent of total plot biomass represented by seeded native ruderal (NR) species per year. Plots where the native ruderal (NR) mix were seeded include NR and NR+BT (*Bromus tectorum* L.). Plots where NR was not seeded include BT, SW (sterile wheat), SW+BT, and US (unseeded). In both years, most plots had no NR species biomass.

Year	Treatment	*% C. album*		*% E. capitatum*		*% H. annuus*		*% S. coccinea*		*% NR (sum)*	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2019	NR seeded	*0.00*	*0.00*	*0.00*	*0.00*	*0.01*	*0.01*	*0.00*	*0.00*	*0.01*	*0.01*
2019	NR not seeded	*0.00*	*0.00*	*0.01*	*0.01*	*0.00*	*0.00*	*0.01*	*0.01*	*0.02*	*0.01*
2020	NR seeded	*0.01*	*0.01*	*0.00*	*0.00*	*0.01*	*0.01*	*0.00*	*0.00*	*0.02*	*0.02*
2020	NR not seeded	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*

I also used non-parametric statistical tests to address the hypothesis that there would be more biomass of NR species in plots where the NR mix was seeded than in plots where the NR mix was not seeded. I analyzed the data separately for 2019 and 2020 using Mann-Whitney U tests with the alternative hypothesis that NR percent biomass in NR-seeded plots was greater than NR biomass in plots not seeded with NR. I used an alpha level of 0.05. In both years, the test failed to reject the null hypothesis (2019 p-value = 0.54, 2020 p-value = 0.93). Based on this test, I did not find support for significantly higher biomass of NR species in plots where the NR mix was seeded. This, combined with the overall very low biomass of NR species in both years, led me to conclude that in answer to Question 3, there is not significant representation of the NR species in the aboveground vegetation community nine and ten years after seeding.

I found no cheatgrass in either year, so I was unable to address questions relating to native ruderal suppression of cheatgrass establishment. In 2019, there were no individuals from the perennial seed mix. In 2020, there was one individual of *Elymus trachycaulus* (Link) Gold ex Shinners and one of *Artemisia frigida* Willd. Neither occurred in a plot where the NR mix was seeded. Thus the 2020 data do not support a facilitation of native perennial species by native ruderal species.

Comparing biomass data from earlier years with 2019-2020 shows that in all but one year more than 90% of biomass was contributed by NR mix species, cheatgrass, and three unseeded species: crested wheatgrass (*Agropyron cristatum*), rubber rabbitbrush (*Ericameria nauseosa*), and bindweed (*Convolvulus*

arvensis) (Figure 1.4). The data show that the NR mix and cheatgrass both comprised varying but higher percentages of total biomass from 2011-2014 when compared to 2019-2020. In 2019-2020, their biomass was mainly replaced by crested wheatgrass.

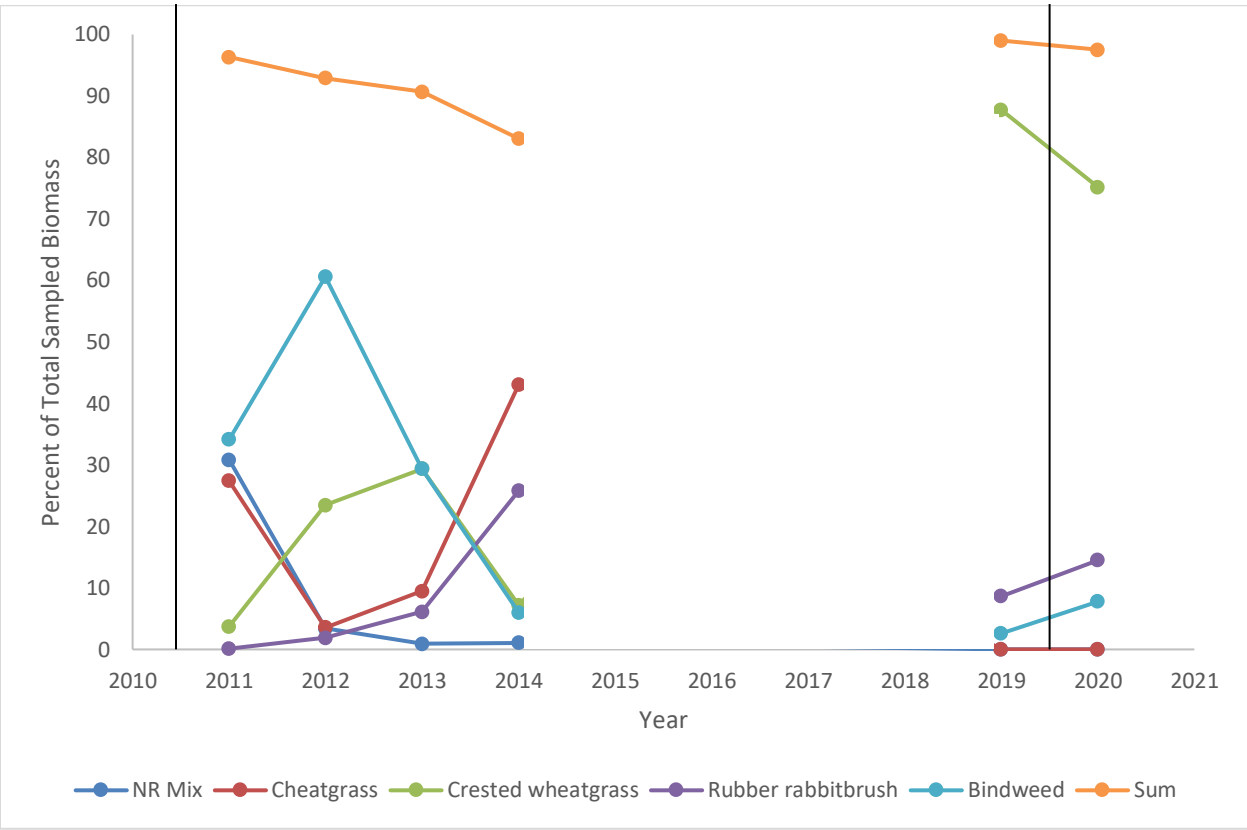

Figure 1.4 Percent of Total Biomass for Major Constituents of Vegetation Community Between 2011-2020. Data were not collected from 2015-2018. See Table 1.1 for composition of the native ruderal (NR) mix. NR mix and cheatgrass were seeded in 2010, while the other species were unseeded. Vertical bars represent soil disturbances (rototilling + soil ripping in 2010, rototilling in 2019). The "sum" points in orange represent the sum of the other five points listed for a given year. In every year except 2014 the species shown represented more than 90% of total biomass sampled.

4. Discussion

Overall, field studies showed there was almost no biomass of seeded species in 2019 or 2020. This included seeded native ruderal species, native perennial species, and cheatgrass. Lack of seeded species biomass in the field prevented me from answering questions 3-5, which dealt with native ruderal suppression of cheatgrass and facilitation of native perennial species. Low NR species biomass in the field undermined my hypothesis of persistent native ruderal soil seed banks.

Very low native ruderal biomass also meant that my results did not support the hypothesis that native ruderal species would have significantly higher biomass in plots where they were seeded. Even more surprisingly, NR species did not reappear after rototilling in 2018. The lack of establishment after disturbance in the field directly contradicts the pilot greenhouse results that supported the presence a persistent native ruderal soil seed bank. Comparing these studies directly is complicated by the different metrics I used (seeds/m^2 in the soil seed bank pilot study and biomass in g/m^2 for the field study). Still, the extremely low biomass I measured in the field suggests low aboveground density of NR species. This suggestion of low NR density is supported by the fact that NR species only established in 3/50 field plots in 2019 and 3/50 field plots in 2020.

The contradiction between greenhouse and field results implies that there is a viable seed bank of certain seeded native ruderal species, but that unknown factors prevented those seeds from establishing in the field. I only collected data in the first growing season after rototilling, and it is possible that future years will show more NR establishment. Other possible explanations for a lack of field establishment include legacy effects and competition from crested wheatgrass, as well as herbivory.

Figure 1.4 shows that in each of the first four years of the study, either the NR mix, cheatgrass, or both were competitive with the species that dominated the site before the study (crested wheatgrass, rubber rabbitbrush and bindweed) in terms of percent of total biomass. However, by 2019-2020, the site had returned to a similar composition to pre-study, with crested wheatgrass by far the most dominant species, rubber rabbitbrush and bindweed forming smaller components of the vegetation, and virtually none of the

seeded species remaining. In other words, the pre-existing vegetation community had a strong resilience to the disturbance and successional changes induced as part of the study.

Crested wheatgrass is known to create legacy effects on sites through plant-soil feedbacks that favor its own growth. Because crested wheatgrass dominated the site before this study was initiated, these legacy effects could explain the difficulty of replacing crested wheatgrass with other species. Prior work has suggested that crested wheatgrass creates plant-soil feedbacks by altering AMF community composition, resulting in decreased AMF colonization of native species (Jordan et al. 2012, Perkins and Nowak 2013). This appears to be a conspecific positive feedback, that improves the growth of plants generally but disproportionately benefits crested wheatgrass over other species (Perkins and Nowak 2013). These disproportionate positive effects allow crested wheatgrass to compete strongly against other plant community members, overriding any improved growth they may have gained from the feedback. Prior studies have found that forbs (Jordan et al. 2008) and annual species (Kulmatiski et al. 2008, Perkins and Hatfield 2014) show the largest growth reductions on soils pre-conditioned by crested wheatgrass. This may explain the low biomass of the native ruderal mix in 2020. Lesica and Cooper (2019) investigated which species might establish successfully in crested wheatgrass stands on the Great Plains despite any plant-soil feedbacks. Of the native forbs seeded in this study, only one (*Sphaeralcea coccinea*) appeared on their list.

Crested wheatgrass suppression of cheatgrass is well established, and managers have seeded it as a buffer against cheatgrass invasion (Whitson and Koch 1998, Blank et al. 2015, Davies et al. 2015). However, prior studies understood suppression to result from crested wheatgrass' competitive abilities rather than soil legacy effects. Crested wheatgrass may suppress cheatgrass by initiating rapid growth early in the year, quickly establishing high root and shoot biomass, producing high levels of litter, and depleting soil water and nitrogen (D'Antonio and Vitousek 1992, Johnson and Aguirre 1991, Bakker and Wilson 2001, Blank et al. 2015). Of course, soil legacy effects and competition are not mutually exclusive mechanisms to explain crested wheatgrass dominance at a site. The legacy effects can favor crested wheatgrass as succession begins, while its competitive traits may allow it to increase density and dominance

at a site over time. Additionally, crested wheatgrass can outcompete other species including cheatgrass and native species over longer periods of time through high tolerance of drought and disease (Bakker and Wilson 2001). Crested wheatgrass is also highly resistant to mechanical removal treatments such as the soil tilling and ripping methods used in this study (Hulet et al. 2010, Fansler et al. 2011, McAdoo et al. 2017). It may regrow from root fragments but also produces a dense soil seed bank and has high germination rates and seedling vigor (Plummer 1977, Henderson and Naeth 2003). The crested wheatgrass soil seed bank does not seem to be a significant factor in this study, because the greenhouse study found a relatively small crested wheatgrass estimated soil seed bank density of 17 seeds/m^2.

Herbivory may have also reduced the establishment of the NR mix after disturbance. Cumberland et al. (2017) used study plots directly adjacent to those in this study and found that first instar *Melanoplus bivittatus* grasshoppers inflicted heavy mortality on seedlings of various native plant species. These included several native ruderal forbs, one of which, *Helianthus annuus*, was also seeded in the NR mix for this study. In this study, herbivory clearly did not prevent NR mix seedlings from forming 31% of community biomass in 2011, after the initial rototilling. Again, though, the initial seeding rate of NR species was orders of magnitude higher than the soil seed bank density in 2017. Herbivory impacts in 2019-2020 would have been proportionally greater with fewer NR seedlings germinating.

To summarize, my initial questions were: (1) *is there still a viable soil seed bank of the native ruderal species*? (2) *are native ruderal species still present in the aboveground vegetation? (3) have native ruderal species suppressed cheatgrass?* (4) *will the presence of a native ruderal soil seed bank limit the re-establishment of cheatgrass after a soil disturbance*? And (5) *have native ruderal species facilitated the establishment of native perennial species?* This study found that there is still a viable native ruderal soil seed bank, but that native ruderal seeds failed to produce significant aboveground biomass even after a soil disturbance. Future work in sites with minimal or no confounding effects from crested wheatgrass would better address this question. There is still evidence from the first two years of the study that the native ruderal species seeded in this study do suppress cheatgrass, and more work on the long-term effects of seeding those species would be helpful. However, the presence of crested wheatgrass is likely also the cause

of the complete lack of cheatgrass in the plots 9 and 10 years after the original seeding, which meant I could not determine whether the presence native ruderal species in certain plots had suppressed cheatgrass in those plots after the initial two growing seasons. What native perennial species did germinate were not in native ruderal-seeded plots. This suggested that there was not a facilitation effect. However, there was very little native perennial biomass, so the results are inconclusive. And finally, a lack of cheatgrass biomass prevented me from addressing whether native ruderal soil seed bank germination after disturbance would minimize cheatgrass establishment.

Initial evidence from this study, as well as from others such as Daehler 2003, Leger et al. 2014, and Barak et al. 2015, may spur land managers to experiment with seeding native ruderal species at sites that are vulnerable to cheatgrass invasion. To successfully implement this technique, they will have to tailor unique native ruderal seed mixes to various sites. Cheatgrass successfully invades ecosystems with a wide variety of soil types, elevations and climates (Monsen 1994), and it may be difficult to find native ruderal species with similar adaptability. The native ruderal species in this study were selected to grow in shortgrass steppe, and different ecosystems would require different species. Although in this study ruderal seeds were rototilled into the soil, a native ruderal soil seed bank could be created by adjusting a rangeland seed drill to the appropriate planting depth. The same species mix could be seeded at two or three depths in multiple passes with the drill in order to promote some initial establishment as well as to create a soil seed bank.

Even if native ruderal species can suppress cheatgrass, seeding them to combat cheatgrass invasion should be used as one tool in a suite of integrated management techniques. Studies have shown that targeted short periods of fall or early spring grazing (when many native perennial species are dormant) may be effective at controlling cheatgrass (Pellant 1990, Schmelzer et al. 2014, Perryman et al. 2020). Historically, overgrazing in the Great Basin has promoted cheatgrass invasion (Mueggler 1972, Mack 1981, Jones and Nielson 1997, Mukherjee et al. 2013). Therefore, it is important to use grazing management practices such as maintaining proper stocking rates and avoiding repeated grazing of the same areas in successive years (Davies and Boyd 2020) when implementing targeted grazing.

Kennedy (2018) showed that certain highly selective deleterious rhizobacteria strains can suppress cheatgrass, as well as *Aegilops cylindrica* Host (jointed goatgrass) and *Taeniatherum caput-medusae* (L.) Nevski (medusahead) without adversely affecting other native or desirable species. However, she found that this method was much more successful (~90%) in mixed invasive-native rangelands when compared to invasive monocultures (~50%). Planting competitive native ruderals into these monoculture situations could boost the success of this method. Various parasitic fungi also infect cheatgrass stands and can cause high mortality. These could also be a useful biocontrol, and they appear to parasitize cheatgrass during different phenological stages to avoid competing with each other and could thus likely be applied in concert as biocontrols (Meyer et al. 2016).

Selective herbicides that have been shown to be effective against cheatgrass include imazapic and the pre-emergent indaziflam, while monotypic stands of cheatgrass respond to the nonselective post-emergent glyphosate (Pyke et al. 2014, Munson et al. 2015, Sebastian et al. 2017a). Repeated herbicide treatments before seed set can quickly deplete cheatgrass' transient soil seed bank, which is typically viable for around 3-5 years (Sebastian et al. 2017b). Other control techniques include late-season controlled burns (Calo et al. 2012, Kessler et al. 2015), and seeding cultivars or ecotypes of native perennial grasses that have adapted to competition with cheatgrass (Leger 2008, Goergen et al. 2011). All of these techniques will likely prove most effective when combined into a comprehensive management plan.

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Chapter 2 - SOIL PRE-CONDITIONING WITH BEARBERRY (*ARCTOSTAPHYLOS UVA-URSI*) AND PERENNIAL GRASSES TO FACILITATE LODGEPOLE PINE (*PINUS CONTORTA*) GROWTH IN PILE BURN SCARS

1. Introduction

1.1 Slash Pile Burning

Forest managers thin forests for a variety of reasons, such as removing wildfire fuels (Rhoades et al. 2015, DeSandoli et al. 2016), improving tree resistance to drought or insect infestation (Cole et al. 2016, Vernon et al. 2018), removing hazardous standing dead material near roads or buildings, improving timber quality by reducing competition (Ballard and Long 1988) and mitigating woody plant encroachment in grasslands (Halpern et al. 2014). To dispose of unneeded timber after thinning projects, managers often pile it together, dry it over the course of a year or two, and then burn the pile. This process is known as slash pile burning, and the resulting de-vegetated area is called a pile burn scar (Figure 2.1). While slash pile burning is one of the cheapest ways to dispose of unsaleable timber (Finkral et al. 2012), it has some negative ecological effects. Because of the density of fuels, pile burning heats soils to high temperatures for extended periods (Halpern et al. 2014). When burning piles composed of large-diameter tree boles, temperatures can exceed 500°C, surpassing the heat produced by even high intensity wildfires (Massman et al. 2008). This high-temperature burning may kill most living seeds and microorganisms in the soil (Korb et al. 2004, Jiménez Esquilín et al. 2007). It may also result in changes to soil physical and chemical structure that can shape the process of recolonization of pile burn scars by plants and microbes. These changes include increased plant-available soil nitrogen and phosphorus (Fornwalt and Rhoades 2011, Creech et al. 2012, DeSandoli et al. 2016), loss of soil porosity (Campbell et al. 1995) and increased hydrophobicity (Hubbert et al. 2015). Decreased porosity and increased hydrophobicity both reduce water infiltration in soil. Several prior studies have noted that the significant effects of slash pile burning on plant communities. Relative to unburned areas, pile burn scars tend to have increased bare ground, decreased

native species richness and cover, and increased exotic species cover (Fornwalt and Rhoades 2011, DeSandoli et al. 2016). However, there are also cases when passively revegetated pile burn scars show similar exotic species cover to unburned areas (e.g. Creech et al. 2012, Rhoades et al. 2015).

Figure 2.1. A pile burn scar sampled at the Fraser site. There is little vegetation regrowth in July 2019, 2-3 years after the pile was burned. Understory vegetation and lodgepole pine seedling regrowth are visibly greater in the unburned area on the far side of the pile. Most vegetation in pile burn scars sampled for this study consisted of ruderal herbs, including several noxious weed species. Mesh tree cages and plastic seed traps shown in the photo are for a separate studies not discussed in this thesis.

On the Arapahoe-Roosevelt National Forest in northern Colorado, lack of lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia*) recruitment after pile burning is a particular concern to the U.S. Forest Service. Lodgepole pine are fire-adapted and at least some populations have serotinous cones (Lotan 1967, Alexander 1974). There is typically significant recruitment within the first five years following wildfire (Harvey et al. 2016). In pile burn scars, however, a lack of lodgepole pine recruitment can persist

for decades (Rhoades and Fornwalt 2015). Several factors might explain the persistent absence of trees in these scars. These include altered soil conditions, high levels of herbivory or seed predation, and competition from perennial grasses (Rhoades and Fornwalt 2015). It is most likely a combination of these factors that limits seedling recruitment. Individual pile burn scars are small; usually less than 5 m but sometimes up to 30 m in diameter in northern Colorado National forests (Rhoades and Fornwalt 2015). However, the practice of pile burning is widespread: in 2015, there were 140,000 slash piles waiting to be burned in northern Colorado National Forests (Rhoades et al. 2015). Cumulatively, pile burn scars may constitute considerable landscape changes. After thinning projects, they can make up >15% of treated areas (Busse et al. 2013).

1.2 Plant-soil feedbacks

In this study, I focused on altered soil conditions as a potential inhibitor of lodgepole pine regeneration in pile burn scars. In particular, I investigated whether establishing understory species in pile burn scar soil could create plant-soil feedbacks that either favored or suppressed growth of subsequently transplanted lodgepole pine seedlings. I attempted to determine whether one or both of two soil pre-conditioning treatments would create positive or negative feedbacks for lodgepole pine growth. These two treatments were pre-conditioning with perennial grass and *Arctostaphylos uva-ursi* (L.) Spreng. (common bearberry). The perennial grass treatment was selected to represent a typical postfire restoration treatment meant to establish cover and reduce erosion.

On the other hand, bearberry and other *Arctostaphylos* species are rarely used in forest restoration. Bearberry is a procumbent evergreen shrub. It is a common understory species in boreal and montane lodgepole pine forests throughout western North America (Crane 1991). The shrub prefers dry and nutrient-poor soils, and grows in exposed, rocky sites or canopy gaps as well as in shaded sites (Kershaw et al. 1998, Nadeau and Corns 2002). Although it is slow-growing, its adaptation to exposed sites with poor soils could make it an effective colonizer of pile burn scars.

There are a variety of plant-soil feedbacks through which understory species might influence subsequent lodgepole pine growth. These feedbacks could be either positive (i.e., understory species affect soil to promote lodgepole growth) or negative (understory species affect soil to hinder lodgepole growth). Some plant-soil feedbacks will not be relevant for a greenhouse experiment lasting only a few months, for example those involving litter chemistry or changes to weathering rates. (Ehrenfeld et al. 2005).

Plant interactions with mutualist soil microbiota, though, could shape lodgepole pine recruitment on the timescales observed in this study. These interactions are likely to be among the most potent plant-soil feedbacks (Ehrenfeld et al. 2005). In this study, the feedback would occur if the pre-conditioning species promoted the establishment and growth of a soil microbial community that subsequently benefitted lodgepole pine growth. Symbiotic root-inhabiting fungi called mycorrhizal fungi are likely candidates to drive such a feedback. Mycorrhizal fungi occur in 94% of vascular plant species and can help plants to access water and various nutrients in exchange for carbon (Grimoldi et al. 2006, Brundrett 2009, Buscot 2015, Soudzilovskaia et al. 2015). Mycorrhizas (the term for the association between the plant and the mycorrhizal fungus) may aid plants in establishing in dry soils (Ehrenfeld et al. 2005, Meijer et al. 2011, Valliere et al. 2016), which could be particularly relevant in pile burn scars.

Perennial grasses are unlikely to promote mycorrhizal facilitation of lodgepole pine. Grasses and pines are generally thought to associate with different types of mycorrhizal fungi: arbuscular mycorrhizal fungi (AMF) for the former and ectomycorrhizal fungi (EMF) for the latter (Borowicz and Juliano 1991, Rydlová and Vosátka 2001). AM fungi do colonize the roots of several species in the family Pinaceae including *Pinus contorta* (Cázares and Smith 1996, Smith et al. 1998, Wagg et al. 2011, Murata et al. 2015). However, in most cases AMF in Pinaceae roots do not form arbuscules, the root intracellular structures that are the sites of nutrient exchange between plants and AMF (Wright 2005), suggesting that most of these AMF associations are not mutualistic. Smith et al. (1998) observed a case where AMF root colonization doubled leaf P of Douglas fir (*Pseudotsuga menziesii*) without the presence of arbuscules. Still, they did not observe any difference in shoot N, aboveground biomass, or belowground biomass between colonized and non-colonized trees.

AMF and EMF have different ecological niches, with the former acquiring mainly inorganic nutrients, especially phosphorus, while the latter aids in decomposing soil organic matter and acquiring organic nitrogen for plants (Frouz et al. 2019). The ecological relationships between AMF and EMF are complicated, but generally seem to be competitive. EMF-associating trees and shrubs have been shown to reduce the abundance and diversity of AMF communities associating with co-occurring herbaceous plants (Knoblochová et al. 2017), while EMF-associated plants have seen reduced growth when grown in soil inoculated with AM fungi, presumably due to competition between EMF and AMF (Osonubi et al. 1991). Thus, it is possible that preconditioning with perennial grasses could suppress ectomycorrhizal activity in lodgepole pine, with potential resultant negative effects on lodgepole growth.

On the other hand, there is evidence that pre-conditioning with bearberry might promote mycorrhizal communities that would also benefit lodgepole pine. *Arctostaphylos* species are not ectomycorrhizal or arbuscular mycorrhizal. Instead, they participate in a third kind of mycorrhiza called an arbutoid mycorrhiza. These symbioses have characteristics of both ectomycorrhizas and arbuscular mycorrhizas. Like ectomycorrhizas, they produce a fungal sheath around the root tips as well as an intercellular Hartig net (Smith and Read 2008). Like arbuscular mycorrhizal fungi, arbutoid mycorrhizal fungi penetrate the epidermal layers of the roots. However, there is no formation of arbuscules. The fungi that form arbutoid mycorrhizas with bearberry also form ectomycorrhizas with various tree species, including *Pinus ponderosa* Lawson and C. Lawson, *Pinus banksiana* Lamb., *Pseudotsuga menziesii* (Mirb.) Franco, and *Picea abies* (L.) Karst. (Visser 1994, Molina et al. 1997, Hagerman et al. 2001, Mühlmann and Gobl 2006). In other words, the same species of fungi vary the structure of the mycorrhizal partnership they form depending on the plant partner.

Bearberry is a generalist mycorrhizal partner (Molina and Trappe 1982, Krpata et al. 2007), which may explain how it associates with mycorrhizal species that are found in so many tree species. Several authors have discussed bearberry as a possible refugium for ectomycorrhizal fungi that could benefit trees through disturbances such as wildfire, clear-cut logging, or landslides (e.g. Visser 1995, Hagerman et al. 2001, Krpata et al. 2007). Furthermore, arbutoid mycorrhizal plants related to bearberry have been shown

to facilitate the growth of trees growing nearby. Horton et al. (1999) found that Douglas fir on the California Coast established only in stands of the arbutoid mycorrhizal manzanita (*Arctostaphylos* spp., same genus as bearberry) rather than the co-occurring arbuscular mycorrhizal shrub *Adenostoma fasciculatum* Hook. & Arn. There was no difference in light, temperature, allelopathy, K, or available N between patches of the two shrubs. However, one year after Douglas fir seedlings were transplanted into *Adenostoma* and manzanita patches, Douglas fir in manzanita patches had higher mycorrhizal colonization and diversity. The majority of mycorrhizal species colonizing Douglas fir in manzanita patches also colonized manzanita roots.

1.3 Questions and Hypotheses

The work of Horton et al. (1999) led me to question whether bearberry, which co-occurs commonly with lodgepole pine, shares mycorrhiza with the pine and thus facilitates its re-establishment after disturbance. I found no literature that addressed these questions among the two papers generated by a Web of Science search for topic keywords "(Arctostaphylos uva-ursi OR bearberry), mycorrhiza, and (lodgepole pine OR pinus contorta). With this study, I examined whether planting bearberry in pile burn scar soils could benefit transplanted lodgepole pine seedlings by encouraging the formation of beneficial mycorrhizal communities from any mycorrhizal propagules that remained after pile burning. The study addressed the following questions:

Question 1**:** What are the effects of soil pre-conditioning with perennial grasses vs. bearberry on lodgepole pine establishment in burn pile scars?

Hypothesis 1: Pre-conditioning soil in burn pile scars with bearberry will improve the relative stem volume growth rate of lodgepole pine relative to pre-conditioning with a perennial grass mix.

Question 2: If there are pre-conditioning effects of bearberry, are these related to mycorrhizas?

Hypothesis 2**:** If lodgepole pine stem volume relative growth rate increases when grown in soil pre-conditioned with bearberry, periodically severing hyphae within the rhizosphere of the lodgepole pine during tree seedling growth will reduce this effect.

2. Study Site and Soil Sampling

To address these questions, I conducted a greenhouse study using soil collected from pile burn scars. I collected soil samples at two sites in north-central Colorado on the Arapahoe-Roosevelt National Forest. One site was located within the Fraser Experimental Forest near the town of Fraser, and the other was along Crown Point Road near Cameron Pass. Both sites are in lodgepole pine forests, at elevations ranging from 2706-3170 m. Slash piles were burned onsite after roadside hazard logging of lodgepole pine killed by mountain pine beetle (*Dendroctonus ponderosae*) outbreaks. Piles were burned between 2016-2017, and their widths range from approximately 15-30 m (Figure 2.1).

I sampled 5 pile burn scars at each site, for a total of 10 piles sampled. Within each pile burn scar, I collected 12 samples every meter along 1-2 transects (smaller piles required 2 transects). The transects passed from the edge of a pile to the center in order to capture spatial variability in the heat of the pile burns, because the highest density of fuels was found at the center of the piles. I sampled to a depth of 5 cm, and combined the 12 samples into a single composite sample for each pile burn scar.

3. First Greenhouse Study

3.1 Study Design and Data Collection

I used the soil samples for two greenhouse studies. The first study addressed Question 1: whether there were any effects of soil pre-conditioning with either perennial grasses or bearberry on lodgepole pine growth. There were three soil pre-conditioning treatments: unseeded, perennial grass mix, or bearberry. The species in the perennial grass mix are listed in Table 2.1. All of the species in the perennial grass mix used in this study are confirmed arbuscular mycorrhizal species except *Bromus marginatus* (Table 2.1). Each of the three pre-conditioning treatments was tested on soil collected from all 10 piles sampled. This meant there were 30 unique treatment-pile combinations. Each treatment-pile combination had 6 replicates, giving a total of 180 experimental units. Each experimental unit consisted of one pot containing a mix of 1% field

soil and 99% non-mycorrhizal potting medium. The potting medium was a mix of equal parts sand, fritted clay, and potting soil.

Table 2.1 Perennial Grass Pre-Conditioning Mix. This mix was selected to represent the grass component of a typical postfire restoration treatment meant to establish cover quickly and reduce erosion. Mycorrhizal data from Monz et al. 1994, Phoenix et al. 2004, Owen et al. 2013, Boldt-Burisch et al. 2018, Serpe et al. 2020

Species	Common Name	Arbuscular Mycorrhizal
Elymus elymoides	Bottlebrush squirreltail	Yes
Elymus trachycaulus var. *trachycaulus*	Slender wheatgrass	Yes
Bromus marginatus	Mountain brome	Unknown
Pascopyrum smithii	Western wheatgrass	Yes
Poa secunda var. *ampla*	Sandberg bluegrass	Yes
Koeleria macrantha	Prairie junegrass	Yes

I created the perennial grass treatment by seeding two each of the constituent species into each pot and removing any duplicates as they germinated so that the result was one individual of each species per pot. I planted extra seeds of the perennial grass mix into separate trays as a source of seedlings to be transplanted into any pots where one or more of the perennial grasses did not establish. I purchased nursery-propagated bearberry seedlings originally from cuttings taken on Grand Mesa, Colorado, and transplanted these into bearberry treatment pots. The pre-conditioning period was 98 days for the bearberry and unseeded treatments, and 112 days for the perennial grass treatment. This allowed the seeds in the perennial grass treatment extra time to germinate and establish, which was not necessary for the transplanted bearberry seedlings.

During the pre-conditioning, I watered regularly and removed any weed seedlings. At the end of the pre-conditioning period, I removed the shoots of all pre-conditioning species from the pots. I then transplanted one lodgepole pine seedling into each pot. The lodgepole pine seedlings came from a seed source in a National Forest in Colorado, and I purchased them from the Colorado State Forest Service. I took initial measurements of lodgepole pine seedling height and diameter as a benchmark for growth. I could not use biomass as a metric since drying for the initial biomass measurements would kill the trees.

Before making the initial measurements, I marked the circumference of the seedling just below the lowest branch, or the lowest needle if there were no branches. I measured diameter at this point, and measured height from this point to the top of the apical bud at the top of the bole. Marking with ink meant I could be sure to measure from the same spot during final measurements. I allowed the lodgepole pine seedlings to grow for 42 days before taking final height and diameter measurements.

3.2 Data Analysis

Before running statistical tests, I converted each pair of height and diameter measurements taken on the same seedling at the same time into an estimated stem volume. For the estimate, I used the stem volume equation for *Pinus contorta* var. *latifolia* from Nigh (2016). This is the only subspecies of lodgepole pine present in Colorado, where my trees were sourced. Nigh (2016) found that this equation had no residuals from observed mean volumes for trees in the smallest volume class. Since the tree seedlings I used in this study correspond to that volume class, the equation should model their stem volumes very accurately. I used the initial and final volumes to calculate a relative growth rate in terms of volume (RGR_v) for each seedling in the study using

$$\frac{\ln(v_2) - \ln(v_1)}{t_2 - t_1}$$

where v_2 is final volume in cm^3, v_1 is initial volume in cm^3, t_2 is final time in days, and t_1 is initial time in days. This is the classical relative growth rate equation from Fisher (1921) with volume substituted for mass.

I conducted all statistical analyses using R. I analyzed data separately from the two locations, Crown Point and Fraser. For each location, the treatment design was nested with two levels. Pre-conditioning treatment (perennial, bearberry, unseeded) was a fixed effect nested within pile (5 piles per location), where each pile served as a random block. RGR_v data were not normally distributed for any pre-

conditioning treatment at either location. Square-root transformations only improved the normality of the bearberry treatment, not the perennial grass or unseeded treatments, so I decided to analyze the untransformed data with a non-parametric test. I used the Friedman test, which employs a matrix of blocks (rows) and treatments (columns) (Friedman 1940). In this case, the blocks were the piles. Since the Friedman test does not allow replication within the blocks, I averaged the six replicates for each pile into a single value. The test assigns ranks to each treatment within a block, and sums the ranks of each treatment. It is then possible to compare the treatments using the summed ranks. For post-hoc pairwise comparisons of treatments, I used Miller's pairwise test (Miller 1981).

3.3 Results

Across both sites, RGR_v rates were between 0.00-24.73 $mm^3/(cm^3*day)$. This corresponded to absolute increases in height between 0.00-0.07 cm/day and increases in diameter between 0.00-0.06 mm/day.

Data for all treatments and both locations were non-normally distributed (Figures 2.2-2.3). At both locations, RGR_v data for the unseeded treatments had distributions that were more heavily left-skewed than other treatments. This indicated that mean RGR_v in the unseeded treatments was probably closer to zero than for treatments where understory species were used to pre-condition soil. Data were also notably left-skewed for the perennial grass treatment at Fraser.

For both Crown Point and Fraser, the Friedman test showed that there were significant differences in RGR_v between the treatments (p-value=0.02 for both locations). The pairwise comparisons showed that only one pair of treatments was significantly different at each location: bearberry-unseeded at Fraser (p-value = 0.02) and perennial-unseeded at Crown Point (p-value = 0.02). In both cases, the pre-conditioned soil (bearberry or perennial grass) produced higher mean lodgepole RGR_v rank than the unseeded treatment soil (Figure 2.4).

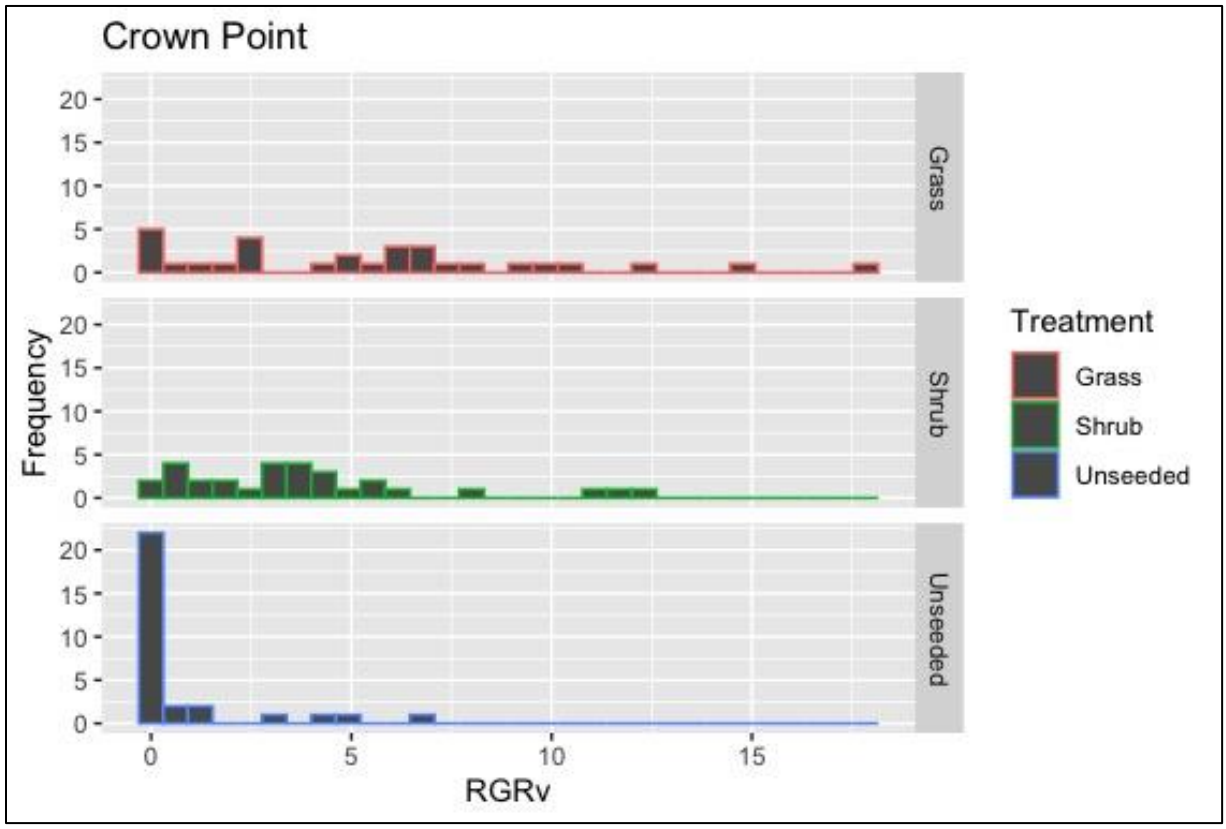

Figure 2.2 Histograms of RGR_v (relative growth rate in terms of volume) values for lodgepole pine grown in soil samples collected from pile burn scars along Crown Point Road, CO. Separate histograms are shown for each soil pre-conditioning treatment. The shrub treatment was pre-conditioned with bearberry. The unseeded treatment produced more zero values, and overall smaller values than the other two pre-conditioning treatments, leading to a left-skewed distribution. RGR_v is calculated using the relative growth rate equation from Fisher (1921) with volume substituted for biomass. RGR_v values are in $mm^3/(cm^3*day)$.

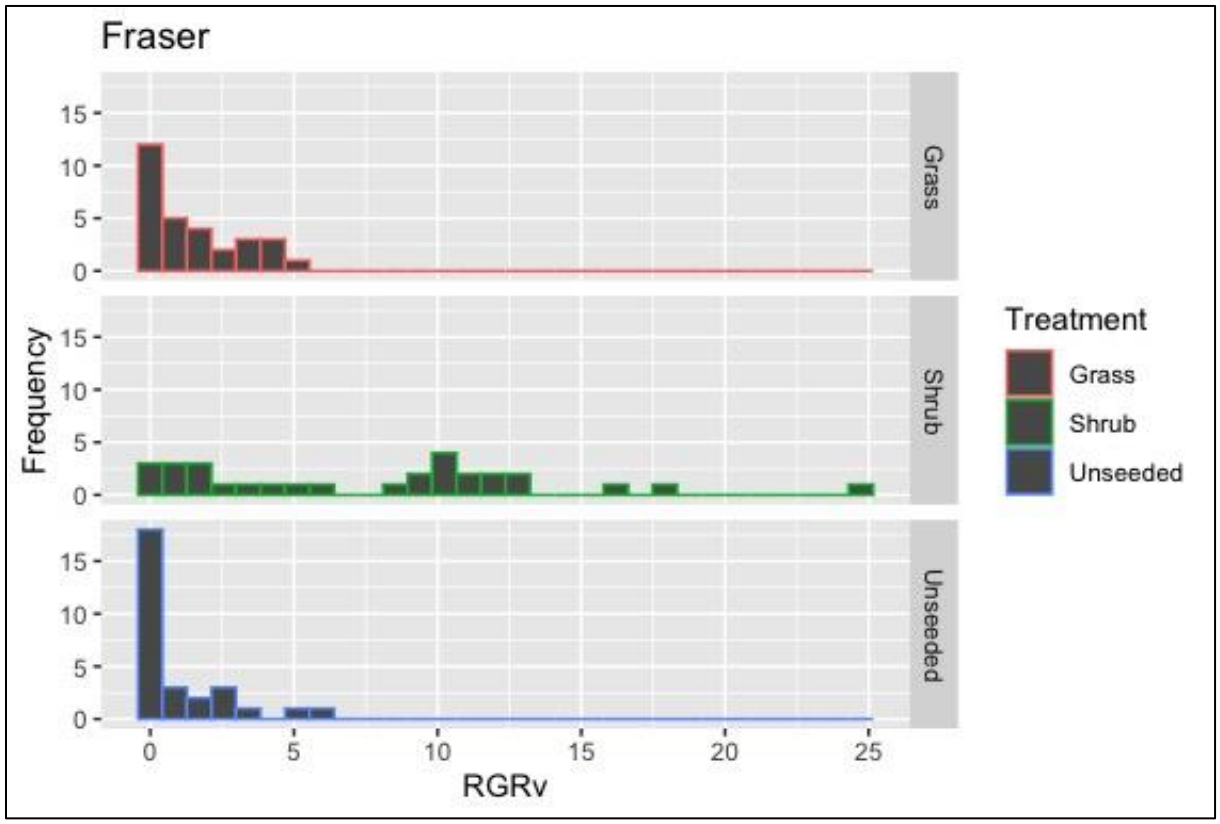

Figure 2.3 Histograms of RGR_v (relative growth rate in terms of volume) values for lodgepole pine grown in soil samples collected from pile burn scars in the Fraser Experimental Forest, CO. Separate histograms are shown for each soil pre-conditioning treatment. The shrub treatment was pre-conditioned with bearberry. Both the perennial grass and unseeded treatments had a significantly left-skewed distribution due to higher numbers of zero values, and overall smaller values, compared to the shrub pre-conditioning treatment. RGR_v is calculated using the relative growth rate equation from Fisher (1921) with volume substituted for biomass. RGR_v values are in $mm^3/(cm^3*day)$.

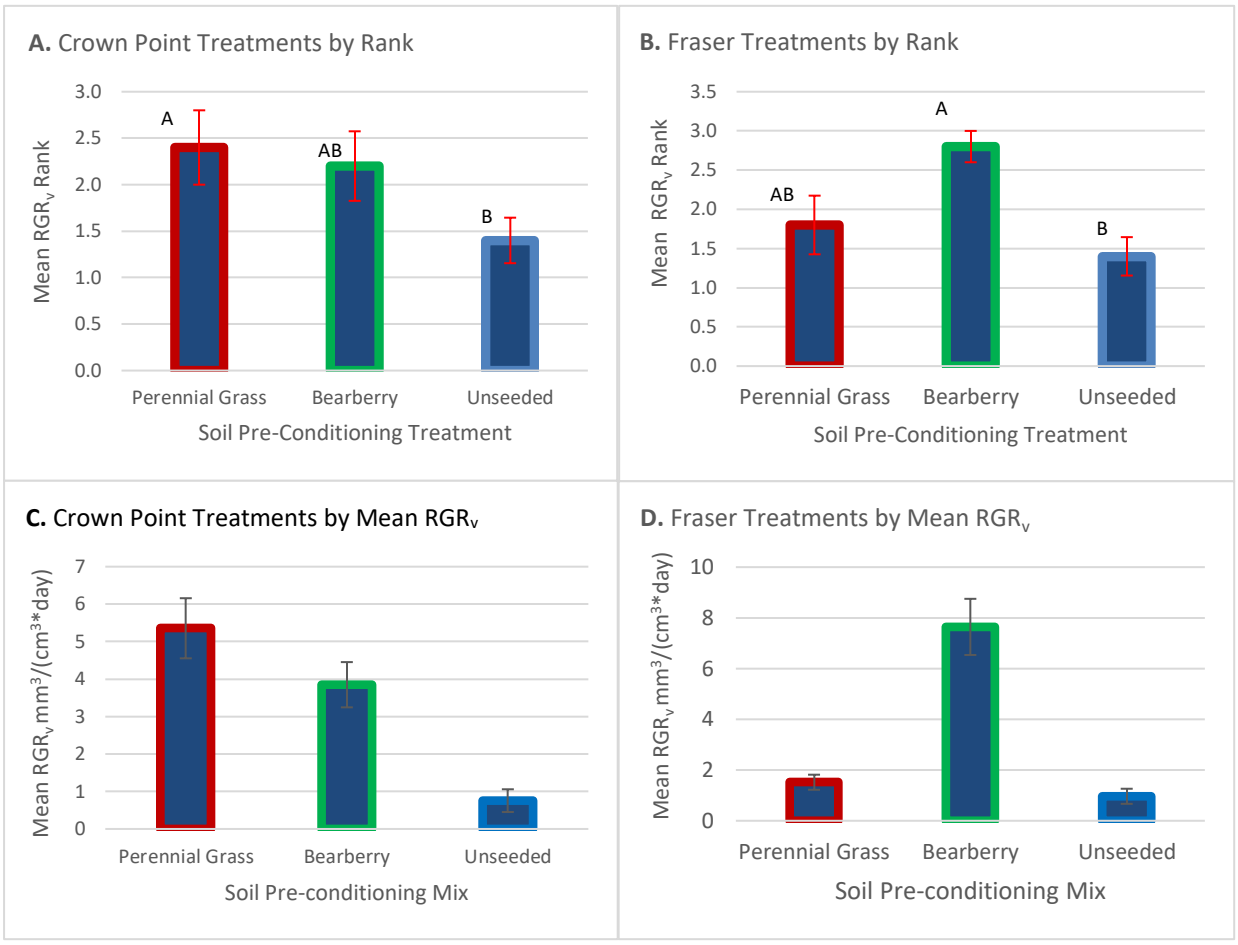

Figure 2.4 Comparisons of lodgepole per pile burn scar by soil pre-conditioning treatment, for soil samples collected along Crown Point Road or in the Fraser Experimental Forest. Panels A and B compare treatments by mean RGR_v rank, with 3 as the highest possible rank and 1 as the lowest. At each location, columns with different letters have significantly different mean RGR_v ranks at $\alpha = 0.05$. Significant differences are based on Friedman tests. At Crown Point, only perennial-unseeded differed significantly, and at Fraser, only shrub-unseeded differed significantly. In both cases the pre-conditioned treatment (either bearberry or perennial grass) showed higher mean RGR_v than the unseeded treatment. Panels C and D compare treatments by mean RGR_v. For each panel, error bars show ± standard error in the units of the y axis.

4. Second Greenhouse Study

4.1 Study Design and Data Collection

I also used soil from the same sites for a second greenhouse study meant to address Question 2. The study aimed to determine whether pre-conditioning with bearberry improved lodgepole pine growth specifically by developing a beneficial mycorrhizal community before the lodgepole was transplanted. This second study was based on the methods described in Weremijewicz and Janos (2019). These methods attempt to determine the effects of symbiotic mycorrhizal networks on plant growth by creating a treatment where mycorrhizal hyphae but not roots are allowed access to an extra reservoir of soil resources. Compared with the first study, there was one fewer pre-conditioning treatment, an additional treatment level for access/no access of mycorrhizas to extra soil resources, and soil used was from 2 piles per site rather than 5 (Figure 2.5). Data from Crown Point and Fraser were still analyzed separately. Because this study was focused on whether or not bearberry affected lodgepole pine growth through common mycorrhizal networks, I did not include a perennial grass treatment. For each pre-conditioning treatment (bearberry or unseeded), mycorrhizal hyphae living within the rhizosphere of the lodgepole pine were assigned to one of two treatments: allowed access to an extra reservoir of soil nutrient (intact), or not allowed access (severed). For this study, I only collected soil from two piles per site: the highest and lowest elevation pile at each site. There were still six replicates of each treatment combination, leading to a total of 2 (locations) x 2 (pre-conditioning treatment) x 2 (mycorrhizal access treatment) x 2 (pile) x 6 (replicate) = 96 experimental units. This treatment design allowed me to address both Questions 1 and 2 in the same study. If Hypothesis 1 was correct, and there was a facilitative effect of bearberry pre-conditioning on lodgepole pine growth, but Hypothesis 2 was not supported, and this facilitative effect was not related to mycorrhizas, then the bearberry treatment would show improved growth in both ‘severed’ and ‘intact’ pots. But if both hypotheses were supported, the bearberry treatment would show improved growth only in ‘intact’ pots, while ‘severed’ pots in the bearberry treatment would show similar RGR_v to pots in the unseeded treatment.

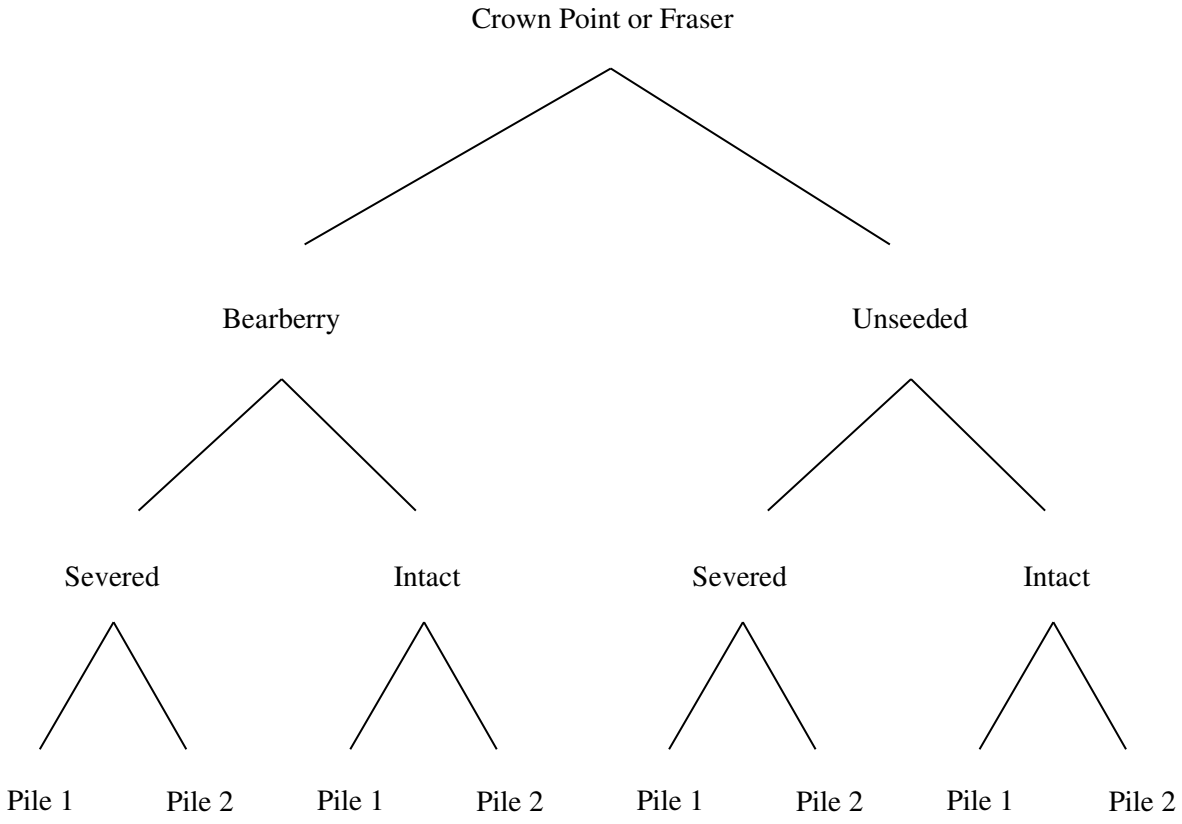

Figure 2.5 Treatment design for the second greenhouse study. Each treatment combination had six replicates, for a total of 96 replicates between the two sites. Soil came from four individual pile burn scars: CP1, CP2, F1, and F2, where CP piles were at the Crown Point site and F piles at the Fraser site. Pile was nested with resource-access treatment. Resource-access treatments were either intact or severed, where intact pots allowed the fungal hyphae within the lodgepole rhizosphere access to extra soil resources and severed pots did not allow this. I hypothesized that extra access to resources would increase lodgepole pine relative growth rate through mycorrhizal symbioses. Resource-access treatment was nested within pre-conditioning treatment, and I hypothesized any extra-resource benefits would be stronger in the bearberry than the unseeded treatment. Data from the two locations (Crown Point and Fraser) were analyzed separately.

In order to create the resource-access treatments, I cut two holes in each of a set of Steuwe and Sons Treepots, which are designed to hold seedling trees, and covered each hole with 40 micrometer mesh. The mesh would allow fungal hyphae but not tree roots to pass through. I then filled each Treepot with the same soil/clay/sand potting medium used in the first greenhouse study, and placed each inside of a larger pot, also filled with the same potting medium. This would allow any plants within the smaller pot to access nutrients in the potting medium from the larger pot only through mycorrhizal networks, not through their own roots. It is possible that nutrients could leach from the larger pot into the smaller pot, but Weremijewicz and Janos (2019) found significantly higher biomass and leaf phosphorus in the intact treatments for a variety of mycorrhizal plant species, suggesting that this study design can elucidate the mycorrhizal reliance of tested plant species.

I transplanted bearberry into half of the Treepots and watered them and removed weeds for a pre-conditioning period of 40 days. The other Treepots comprised the unseeded treatment. At this point, I removed the aboveground biomass of bearberry and transplanted lodgepole pine seedlings into all pots. These lodgepole pine seedlings were sourced from the San Isabel National Forest in south-central Colorado. Just as in the first greenhouse study, I took initial measurements of lodgepole height and diameter. I created the two resource-access treatments: intact mycorrhizal network and severed mycorrhizal network. For each pre-conditioning treatment, half of the seedlings were in the intact treatment and half in the severed. I rotated the Treepots in the severed treatments 360° within the larger pots weekly. This severed any hyphae extending from the lodgepole root network out into the larger pots. After severing, I watered the pots thoroughly to reestablish connections between potting media inside and outside of the Treepots and prevent unintended soil aeration, as recommended by Weremijewicz and Janos (2019). I did not rotate the Treepots in the intact treatment. After 40 days, I took final measurements of lodgepole pine height and diameter and removed lodgepole pine from the pots.

4.2 Data Analysis

Like in the first study, I used height and diameter measurements to calculate an initial and final volume for each seedling based on the lodgepole pine equation from Nigh (2016). I then calculated RGR_v using the RGR equation from Fisher (1921) with volume substituted for biomass.

I again analyzed RGR_v data separately for the two locations, Crown Point and Fraser. Shapiro-Wilk tests showed that the data for Fraser were normally distributed using an alpha level of 0.05. This was not the case for Crown Point. However, after square root transformation Crown Point data were normally distributed according to the Shapiro-Wilk test and using α=0.05. From this point, I analyzed untransformed Fraser data and square root transformed Crown Point data.

I created a linear mixed effects model for RGR_v data from Fraser using the R packages lmerTest and pbkrtest. The model was fitted using the Restricted Maximum Likelihood (REML) method, with Welch-Satterthwaite unequal variance t-tests for comparing fixed effects treatments. The treatment design was again nested, with two fixed effects (pre-conditioning treatment and mycorrhizal treatment) nested within pile, which was treated as a random block.

Using the square root-transformed Crown Point RGR_v data, variance due to pile as a random effect was very small ($3.77 * 10^{-17}$), which indicated that including the random effect was leading to an overfitted model. Therefore, I pooled the blocks for Crown Point and created a linear model with pre-conditioning treatment and mycorrhizal treatment as fully crossed fixed effects.

I evaluated treatment differences for these two models (linear mixed effects model for Fraser and multiple linear regression for Crown Point) using two-way Analysis of Variance (ANOVA) with degrees of freedom approximated using the Kenward-Roger method.

4.3 Results

Across both sites RGR_v for lodgepole pine ranged from 0.00-9.63 mm^3/cm^3*day. This corresponded to absolute increases of 0.00-0.03 mm/day in diameter and 0.00-0.02 cm/day in height.

Using the linear mixed effects model for Fraser, an ANOVA did not show significant differences (α=0.05) in RGR_v among any the fixed effects treatments. These included the pre-conditioning treatments (treatments: bearberry, unseeded; p=0.29), mycorrhizal treatments (treatments, intact, severed; p=0.25), and the interaction of pre-conditioning and mycorrhizal treatments (treatments, bearberry.intact, bearberry.severed, unseeded.intact, unseeded.severed; p=0.89). Thus, the Fraser data do not support Hypotheses 1 or 2.

An ANOVA using the linear model for Crown Point data showed that only the interaction of preconditioning and mycorrhizal treatments, but not the treatments themselves, produced significant differences in RGR_v at α=0.05. However, a post-hoc analysis using Tukey's range test for pairwise comparisons between treatment combinations did not produce any significant differences for any pairs at α=0.05. The test for the bearberry.intact-bearberry.severed treatments did have a much lower p-value than other pairs (Table 2.2), indicating that this pair likely contributed to the significant treatment differences seen in the ANOVA.

Based on the Crown Point data, there is not support for the Hypotheses 1 and 2. However, there is a nonsignificant trend towards lower mean RGR_v in the bearberry.severed treatment when compared to the bearberry.intact treatment, indicating that the bearberry treatment may have facilitated the development of mycorrhizal fungal community that subsequently partnered with lodgepole pine.

5. Discussion

A broad conclusion from the first greenhouse study is that pre-conditioning soil with understory species may benefit lodgepole pine, but that the pre-conditioning species that best facilitate lodgepole growth vary with site. There is evidence from the literature that soil nutrient content can influence the

strength of mycorrhizal symbioses even if the plant and mycorrhizal fungi species involved are held constant (e.g. Van der Heijden and Kuyper 2001). If such an effect occurred in this study, a closer inspection of soil from Crown Point and Fraser sites would be required to better understand how variance in soil conditions might be affecting the results of pre-conditioning. I did not perform such analyses.

Table 2.2 Pairwise comparisons of least squares means of RGR_v for all possible combinations of pre-conditioning * mycorrhizal treatment for Crown Point. Comparisons were done using Tukey's range test. RGR_v is relative growth in terms of stem volume for lodgepole pine, calculated using the equation for RGR from Fisher (1921) with volume substituted for biomass. Bearberry and unseeded are pre-conditioning treatments, while severed and intact treatments refer to the hyphal networks of fungi in the rhizospheres of lodgepole pine used in the study. The data were normalized by square root transformation before statistical analyses. No pair was significantly different at α=0.05, but the bearberry.intact-bearberry.severed pair did have a notably smaller p-value than other treatment pairs.

Treatment Pair	Estimated Difference in Least Squares Mean	Standard Error	Degrees of Freedom	T-ratio	P-value
Bearberry.Intact-Unseeded.Intact	0.52	0.33	44	1.55	0.42
Bearberry.Intact-Bearberry.Severed	0.80	0.33	44	3.00	0.09
Bearberry.Intact-Unseeded.Severed	0.25	0.33	44	0.74	0.88
Unseeded.Intact-Bearberry.Severed	0.28	0.33	44	0.85	0.83
Unseeded.Intact-Unseeded.Severed	-0.27	0.33	44	-0.81	0.85
Bearberry.Severed-Unseeded.Severed	-0.55	0.33	44	-1.66	0.36

Since the literature does not suggest that AMF increase lodgepole pine growth, it is unlikely that mycorrhizal facilitation was the mechanism of increased lodgepole growth in the perennial grass pre-conditioning treatment at Fraser. Root exudation of nutrients might be another feedback to examine in the case of perennial grass facilitation of lodgepole pine growth. Previous studies have observed root exudation of carbon and nitrogen as plant soil feedbacks (Ehrenfeld et al. 2005, Mehrabi et al. 2015, Orozco-Aceves et al. 2017, Lepinay et al. 2018). Although this type of feedback has a small spatial extent, the C:N ratios

of plant exudates directly influence microbial decomposition rates, which in turn determine plant nutrient availability (Ehrenfeld et al. 2005). Nutrient exudation might affect soil conditions enough to change plant growth rates on the short time scales of this study. Examining C:N ratio in soils from Crown Point and Fraser both before and after pre-conditioning with various species could elucidate whether there is support for this hypothesis.

In soil from Crown Point, data from the first greenhouse study did show evidence for the hypothesized benefits of bearberry pre-conditioning for lodgepole pine growth. However, this was contradicted by a lack of difference in lodgepole pine growth between bearberry and unseeded treatments for Crown Point soil in the second greenhouse study. One possible explanation is that the pre-conditioning period was only 40 days in the second study compared with 98 days in the first. A pre-conditioning period longer than 40 days may be required to see the effects of mycorrhizal plant-soil feedbacks. Perhaps the non-significant trend towards a difference between bearberry.severed and bearberry.intact using Fraser soil would have become significant with a longer pre-conditioning period. Allowing longer growth periods for lodgepole pine in a future study would also be preferable if possible. RGR varies widely within a single plant over the course of its lifespan (Hunt and Lloyd 1987), so repeated measures over a longer period of time would be a better way to compare individuals.

Still, based on the second greenhouse study I cannot conclude that there is support for bearberry facilitation of lodgepole pine growth through cultivation of mycorrhizas. There was no significant evidence that bearberry pre-conditioning improved lodgepole pine growth in soil from either location, even taking into account the nonsignificant trend that bearberry.intact had higher RGR_v than bearberry.severed. Rather, this trend indicated that lodgepole pine in soil conditioned with bearberry might have been more reliant on mycorrhiza to produce the same level of growth as lodgepole in the unseeded treatment.

If future studies find further support for soil pre-conditioning as a benefit for lodgepole pine recruitment in pile burn scars, these findings should be integrated with studies on herbivory and competition when pre-conditioning is employed in the field. Managers will need to know whether pre-conditioning with understory species attracts herbivores to lodgepole pine once it is transplanted, and whether or not pre-

conditioning species need to be removed before transplanting pine seedlings in order to reduce competition. Even if soil conditions do prove to be a significant limitation to lodgepole pine growth in pile burn scars, and greenhouse studies show that pre-conditioning has an ameliorating affect, it may be that other limitations will need to be addressed before seeing results in the field.

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